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A MACROINVERTEBRATE BIOASSESSMENT OF TWO STREAMS ON TURNBULL NATIONAL WILDLIFE REFUGE (TNWR)

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A MACROINVERTEBRATE BIOASSESSMENT OF TWO STREAMS ON TURNBULL NATIONAL WILDLIFE REFUGE (TNWR)

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By

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Abstract

This project focused on highly impacted streams at Turnbull National Wildlife Refuge, Cheney, WA to evaluate the health status and ecosystem integrity of the water bodies. Turnbull National Wildlife Refuge contains over 3,000 acres of wetlands, and provides high quality habitat for migrating and breeding waterfowl. However, these wetlands are impacted by both high nutrient levels (Davidson and Rule 2006) and invasive fish (Scholz et al. 2003). This project focused on stream sections of the Company Ditch (CD) and Pine Draw (PD) watersheds. Company Ditch historically had very high nutrient levels and low water quality. Water quality may be improving since closure of a nearby dairy operation in 2008. Pine Draw includes several springs and the only perennial stream habitat on TNWR. This stream has high densities of invasive brook stickleback, and experiences nutrient loading from an unknown source.

Both streams were assessed using benthic macroinvertebrate surveys in 2007, 2008, 2010, 2011, 2013, and sampled again 2016. Benthic macroinvertebrate communities are widely used in monitoring ecosystem health. Physical habitat characteristics and water quality parameters were also measured. Water quality clearly improved in the Company Ditch during 2008-2011 following the closing of the dairy. Water quality has declined in one seasonal Pine Draw site, but remained more consistent in the permanent Pine Draw sites.

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Objective

This study assessed changes through time in the water quality and ecosystem integrity of two streams on Turnbull National Wildlife Refuge (TNWR), Cheney, WA over a 10 year period (2007-2016) through monitoring of benthic macroinvertebrate communities. The assessment includes both seasonal and permanently flowing stream sites. Potential anthropogenic impacts differ between the watersheds and have changed through the study period. In addition to determining trends through time, I investigated how the timing of sampling affected study results, by comparing spring (April/May) and fall (September) macroinvertebrate communities for a subset of the stream sites.

Introduction

Biotic communities react to changes in aquatic habitats and water quality ensuing from anthropogenic disruption, resulting in changes to abundance, diversity, community composition, and life history traits of organisms. The biotic community is defined as a collection of species living in one place (Fishelson et al. 2002; Alsfeld et al. 2008). Bioassessment techniques use information about communities of macroinvertebrates or other stream organisms to evaluate the health status of the stream ecosystems (Bonada et al. 2006). If there are environmental elements that cause pollution or other factors that alter the living conditions of an ecosystem, intervention may be needed to maintain suitable habitat for native communities (Kennish, 1998; Caley et al. 1996, Mirto and Danavaro 2004). Barbour et al. (1999) define bioassessment as an evaluation carried out to determine the state of a water body like a river, stream or a lake, by biological surveys and other direct measurements of the resident biota in surface water. These assessments can be achieved through the examination of the compartments of the ecosystem such as fish, benthos, phytoplankton, and others (Borja, Muxika, and Franco, 2003; Xu, Choi, Yang, Lee, and Lei, 2002).

A change in the physicochemical factors and existing habitats causes a resulting shift in invertebrate composition. The quality of water and biotic composition of rivers and streams shows a mixture of anthropogenic, physical and chemical activities taking place at a catchment area (Sharma and Chowdhary, 2011). Some examples of anthropogenic impacts to aquatic environments that alter macroinvertebrate communities, and that can be detected in bioassessments include: hydrological changes, physical alterations like urbanization and habitat disturbances, and a wide range of pollutants such as chemical runoff, sediment, and excess nutrients (Chatzinikolaou et al., 2006).

Studies by Karr (1991) also establish that both natural and anthropogenic features of river habitats can affect macroinvertebrate community structure. Examples include quantity and quality of food resources; quality of the dwellings like the river bed structure; flow structure such as the level of occurrence and strength of disturbances caused by storms; the quality of water, and biotic relations. Monitoring ecological integrity is needed at regional and national scales to study environmental conditions, but there are some methodological issues with biomonitoring (Carlisle and Meador 2007). For instance, there is little uniformity in how the measurements of biological assemblages are collected, analyzed, and interpreted (GAO, 2002). Indicators are needed for biomonitoring programs across large spatial scales, and these indicators should be relatively simple to collect and broadly interpretable (NRC, 2000). According to Bickham, et al.

(2000), bioindicators are species that have the ability to reveal the aquatic quality or status of an ecosystem. Examples are copepods, which are tiny marine crustaceans that reveal the changes or alterations in the ecosystem. Statzner, et al. (2001) also observed that monitoring such species through physiological, biological, behavioral, and biochemical means will help indicate how much alteration or damage have occurred. In other words, these species can provide more accurate quantitative data. After acquiring these essential data, biomonitoring technicians and biologists are set to find solutions to bring back the health of the body of water and the life in it. Monitoring does not stop after determining the causes and effects. As a matter of fact, it is a long term process that needs adequate funding, equipment, and skills (Conti, 2002; Atalah and Crowe, 2012).

Biomonitoring has two types namely: (1) surveillance before and after the habitat alteration, and (2) ensuring the compliance with standard regulations (Roberts, et al. 2008). It is important to conduct a thorough surveillance before and after the alteration to ensure the existence of species that serve as the indicators to pollutions and physiological and biochemical alterations. On the other hand, it is also important to ensure that the procedure follows bioethical considerations and the standard biomonitoring regulations. All types of biomonitoring are done to maintain water quality (Wells, et al. 2001).

As defined by Rosenberg and Resh (1993), biological monitoring is a way of accessing ecosystem change over time; effects of water quality in the aquatic life stream habitat; point source inputs and other natural and anthropogenic factors. The cornerstone of many biomonitoring programs on the ecological integrity of various streams is the use of benthic macroinvertebrate (structure and function) metrics. Benthic macroinvertebrates are used as indicators of ecosystem health, and descriptors of species-environment relationships (Flinders et al. 2015). The most commonly used methods are based on assessment of fish and macroinvertebrate community metrics rather than periphyton (Association of Clean Water Administers 2012). Changes that occur in the water quality compromise aquatic biodiversity, and the use of benthic macroinvertebrates (species, families, or communities) allows for a full assessment of the ecological effects caused by various pollution sources (Bonada et al., 2006). According to Obolewski (2014), species diversity and richness are indicators that are most likely to be affected by changes of water quality. Biotic and Saprobic indices are effective in assessing the ecological integrity and have been proven useful to biomonitoring program of water quality and aquatic ecosystem since the early twentieth century (Kolkwitz & Marsson, 1909). Biotic indices summarizing responses of specific invertebrate taxa to environmental changes have been formulated mainly in developed countries such as Australia, the United States, and some countries in the European Union (Rosenberg & Resh 1993, Thorne & Williams, 1997). The use of macroinvertebrates in biological monitoring is valuable because measuring water quality (physico-chemical elements) and microbiological components only reflect conditions at the moment of sampling, and may not reflect all human impacts (Armitage et al., 1983).

Some of the most common biomonitoring methods include biotic indices (a scale for showing the quality of ecosystem health by indicating the types of biota that live in it), diversity (a technique that estimates an environment based on diversity of macroinvertebrate species), multivariate approaches (a method that determine more than one variable at a time), multimetric approaches (rely on predictable pattern of tolerance of different species to disturbance gradients), multiple biological traits (e.g. life history, behavior, size, life span) , and functional feeding groups (identifies organisms by their primary food type) (Balsamo, et al. 2012). Among these methods, the multimetric approaches and the biotic indices (including the HFBI which is the model used in this study) are the most commonly used by biologists to evaluate or assess the quality of marine health (Li, et al. 2010; Davies and Tsomides, 2002).

In this study I used a biotic index (HFBI), and life history traits of macroinvertebrates in assessing stream conditions. I also included the degree of dominance of the community by a single invertebrate taxon. Streams with poor conditions that are difficult for many species to persist in are more likely to be dominated by one or a few species. The Hilsenhoff Family Biotic Index (HFBI) incorporates each taxon's pollution tolerance levels, and is particularly sensitive to organic pollution or other conditions that reduce oxygen levels such as eutrophication, sedimentation, or excessively warm water. The percent of the community made of up multivoltine individuals was included because highly disturbed communities typically have a high proportion of multivoltine species, which can quickly respond to disturbance due to short life cycles. Finally, because the stream sites are all highly connected to wetlands and include both seasonal and permanent streams, the proportion of the community made up of predominantly wetland species was included to potentially monitor effects of changes to hydrology over time.

Study Site

The aim of this study is to assess changes through time in the water quality and ecosystem integrity of two streams at Turnbull National Wildlife Refuge (TNWR), Cheney, WA (Figure1). The study streams, Company Ditch and Pine Draw, are located on the eastern edge of the Columbia Basin, 10 km south of Cheney, Washington, Spokane Country. TNWR was established in 1937. It is known for unique wetlands that provide high quality habitat for many wildlife species. TNWR encompasses around 18,207 acres. According to TNWR, birds are the most numerous group of vertebrate wildlife with over 200 species using the refuge, 124 of these species nest there including several waterfowl species, marsh birds, shorebirds and songbirds.

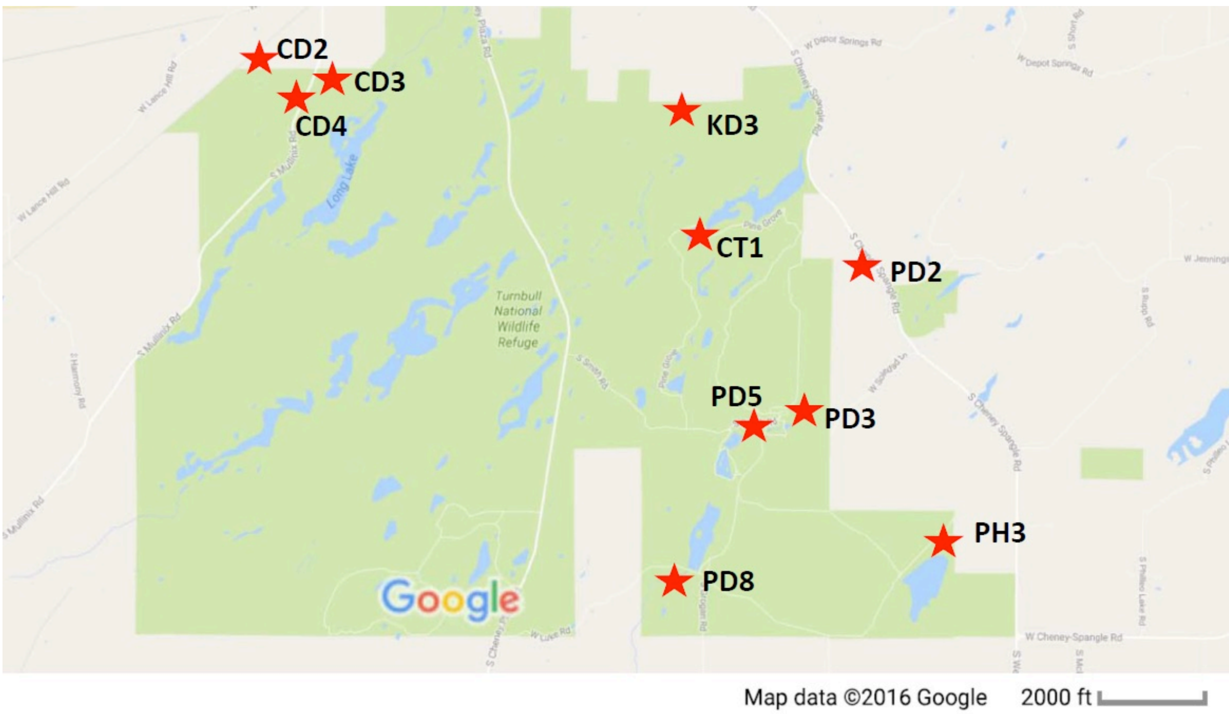

Figure 1. Map of Turnbull National Wildlife Refuge 2017 study sampling sites. Spokane County, WA, USA: Pine Draw (PD2, PD3, PD5, and PD8), Kagele Ditch (KD3); both drain into the Rock Creek watersheds, Company Ditch (CD2, CD3, and CD4); near intersection of Lance Hill and Mullinex Roads, Philleo Drainage (PH3), and Keppel Lake drainage (CT1).

TNWR is within the Channeled Scablands of Eastern Washington, a landscape that was formed 15,000 years ago by ice age floods. These floods scoured out depressions that have become sloughs, seasonally-filled potholes and wetlands. With 3,000 acres of wetlands, TNWR contains wetland communities that provide excellent habitat and refuge for migratory waterfowl (https://www.fws.gov/refuge/Turnbull/, accessed November 2016). Waterfowl require diverse and abundant invertebrate prey communities that help reduce foraging effort while nesting, promote rapid growth for the young, and provide a diet that supports metabolically expensive migration (McNally 2004; Jonathan et. al. 2005; Thongwittaya 2007). These wetlands are impacted by both high nutrient levels (Davidson and Rule, 2006) and invasive fish (Scholz et al. 2003).

Company Ditch has suffered from high nutrient levels and low water quality (Davidson and Rule 2006, McNeely et al. 2007). A nutrient assessment in 2002 found that nutrients entering into the stream feed the growth of bacteria, algae, and fungi causing reduced oxygen level that threaten macroinvertebrate life (Davidson and Rule 2006). The mean concentration of all nutrients in the Company Ditch drainage was 5 times greater than the outflow of control wetlands and Pine Draw and Keagle Ditch drainages (Davidson and Rule 2006). However, water quality may be improving since the closure of nearby dairy operation in 2008 (Bridges et al. 2010). Pine Draw has high densities of invasive brook stickleback and unknown nutrient sources. Within the Pine Draw stream channel, soluble reactive phosphorous, nitrate, total phosphorous, and ammonium levels increased with distance downstream, indicating inputs from an unknown source entering between wetlands.

Ten sites on TNWR with streamflow during spring runoff were sampled. Most were in the Company Ditch or Pine Draw drainages. Sites included 3 locations in the Company Ditch system, CD2, CD3, and CD4, and 4 locations in the Pine Draw system, PD2, PD3, PD5, and PD8. Of these, the 6 sites CD2, CD3, CD4, PD2, PD5, and PD8 were sampled by the EWU Freshwater Invertebrates course in 2007, 2008, 2010, 2011, 2013, and 2016. In each of these years, sites were sampled in late April and early May. In May 2016, 4 additional sites were sampled using identical methods. One of these sites (PD3) was within the Pine Draw watershed. To provide better external reference for these two drainages, one site in the Kaegle Ditch system (KD3), one site in the Philleo Ditch drainage (PH3), and one site in the Keppel Lake drainage (CT1) were also included. The 2002 water quality survey of TNWR found good water quality at the Keppel site (CT1) and poor water quality in the Kaegle and Philleo systems (Davidson and Rule 2006). To evaluate the effects of seasonality of sampling on bioassessment results, the 3 sites that remained wetted at the end of the summer, PD3, PD5, and PD8, were resampled in September 2016. All study sites are indicated on Figure 1. Previous studies by Eastern Washington University (EWU) Freshwater Invertebrate Zoology found a more robust community of macroinvertebrates in Pine Draw, compared to Company Ditch, suggesting better water quality and higher ecosystem integrity (McNeely et al. 2007, Bridges et al. 2010). These studies recommended that an upstream Pine Draw site (PD2) might serve as a reference site for the Company Ditch sites in future assessments, as these sites all had seasonal flow, but PD2 had higher water quality and fewer upstream anthropogenic impacts.

<u>Specific Questions</u>

This study aims to answer the following questions:

1. **Is there a significant difference between the seasonal sites (PD, CD2, CD3, CD4) and**

the permanent sites (PD5 and PD8) in macroinvertebrate indicators of water quality?

2. **Is there a difference among sites within the seasonal category in macroinvertebrate indicators of water quality? Is there a difference between the two sites within the permanent category in macroinvertebrate indicators of water quality?**
3. **Are there year-to year differences? Are there interactions between sites and year?**
4. **How do results of spring monitoring differ from results of fall monitoring?**
5. **How do macroinvertebrate indicators of water quality in the 6 sites monitored since 2007 compare to other stream sites within TNWR?**

Methods

Macroinvertebrate samples, water quality parameters, and data on habitat characteristics were collected over a 100 m reach at each site. The water quality parameters DO (dissolved oxygen), temperature, conductivity, and pH were measured using a YSI 556 handheld multimeter. Samples for determination of dissolved nutrient concentrations were filtered with a 0.7 μm glass fiber filter into acid-washed bottles. Prior to 2013, samples were analyzed for soluble reactive phosphorous using the molybdate method (American Public Health Association 2010) and for ammonium according to the Holmes method (Holmes et al. 1999, Taylor et al. 2007). Samples from 2013 and 2016 were analyzed for soluble reactive phosphorous, ammonium, and nitrate using an Alpkem 3 Flow Analyzer (OIA 2000, 2009a, 2009b). Habitat characteristics and physical parameters included substrate composition, water depth (cm), water velocity (cm/s), width of riparian zone (m), percent canopy cover, dominant riparian plants, and stream discharge. Stream discharge was determined using the X-sectional area method (Gore 2006) using a Marsh-McBirney 2000 or Hach FH950 flow meter. Due to flow meter malfunctions and flows that were sometimes too low to measure accurately, discharge data are not available for all sites in some years.

Macroinvertebrate sampling procedures were modified from the EPA Rapid Bioassessment Protocol III (Barbour, et al. 1999). The most significant modification is that 5 replicate samples, rather than a single sample, were collected from the 100 m study reach at each site. Each study reach was divided into five 20 m sections, and a single, qualitative benthic sample was collected from each section by a single sampler sampling all habitats present for 15 min with a D-frame net. Samples were preserved by adding 95% EtOH and were kept chilled (13 C) until sorting. Random subsamples of each qualitative sample were sorted to remove invertebrates from matrix material including plant detritus and sediments under a dissecting microscope at 10x. Sorting continued until at least 100 specimens were obtained (Barbour et al. 1999). Some subsamples ultimately contained fewer than 100 invertebrates due to sorting errors (plant material initially identified as invertebrate, for example). Initial sorting and identification was performed by EWU Freshwater Invertebrate students, Camille McNeely, and Sultan Areshi. Samples identified by Freshwater Invertebrate students were confirmed by the instructor, C. McNeely. Most invertebrates were identified to Family, but some groups were identified only to Order. Identified invertebrates were scored according to their life history strategy (multivoltine or univoltine) and whether their primary habitats were wetlands or streams (Thorpe and Covich 1991, Merritt, Cummins, and Berg 2008).

Data Analysis

For statistical analysis, I used ANOVAs (Analysis of Variance) as well as logistic regressions to test for differences among sites and dates in water quality parameters and macroinvertebrate community indicators. These analyses were performed in R (R Core Team, 2016). The water quality parameters (dependent variables) analyzed include DO, temperature, pH, and conductivity. Macroinvertebrate community indicators (also dependent variables) include proportion dominant (the proportion of the community made up of the single most common taxon), proportion wetland species, proportion multivoltine, and the Hilsenhoff Family Biotic Index (HFBI). HFBI is calculated according to Hilsenhoff (1988).

These metrics was calculated for each sample. Means, standard deviations, and standard errors were calculated for each site on each date.

Table 1. Calculation of Hilsenhoff Family Biotic Index, and interpretations of HFBI scores. From Hilsenhoff (1988).

$$\boldsymbol{HFBI = \sum (\text{score taxon} \times \#\ \text{taxon}) /\ \text{total}\ \#}$$

Family Biotic Index	Water Quality	Degree of Organic Pollution
0.00-3.75	Excellent	Organic pollution unlikely
3.76-4.25	Very Good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly Poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.00	Very Poor	Severe organic pollution likely

http://cfb.unh.edu/StreamKey/html/biotic_indicators/indices/Hilsenhoff.html

Three subsets of the data were analyzed separately. First, data from the 6 primary sites were analyzed with site and year as independent variables. HFBI was analyzed using 2-way ANOVAs. Frequencies of the dominant taxon, multivoltine individuals, and wetland species were analyzed using logistic regression. These analyses determined if there have been significant changes to water quality and ecosystem integrity over time and if these changes have affected some sites differently than others. This data was also used to compare the Company Ditch and Pine Draw watersheds, and to compare permanent and seasonal sites. A second analysis used 1-way ANOVAs and logistic regressions to examine variation among all 10 sites sampled in May 2016. Finally, 2-way ANOVAs and logistic regressions with site and season as independent variables were used to analyze data for the 3 sites sampled in May and September 2016. Data for all analyses were tested to determine if they met the assumptions of equality of variances and normality.

Results

The results for physical and chemical measurements of water quality are presented first, followed by macroinvertebrate indicators. For each, I first present data for the 6 sites that were monitored over 2007-2016 (Annual Comparisons). I then present data comparing spring and fall 2016 for 3 sites that remained wetted through the early fall. Finally, I will present data for all 10 sites sampled in spring 2016.

Physical Stream Parameters

Annual Comparisons, 2007-2016

Stream water temperatures were coldest in 2011, and warmer in 2007, 2013 and 2016 (Figure 2). The lowest level of mean temperature was recorded in the year 2011 at the site CD4 (mean 7.89, SD 0.07). The highest mean temperature was recorded at CD2 during 2016 (mean 26.31, SD = 1.03). The largest variations among different sites were found during the years 2013 and 2016. Sampling took place in the last week of April and first week in May in all years, so these differences reflect annual variation for this time period. In the Company Ditch, temperature appeared to decline with distance downstream from CD2 to CD4 beginning in 2011.

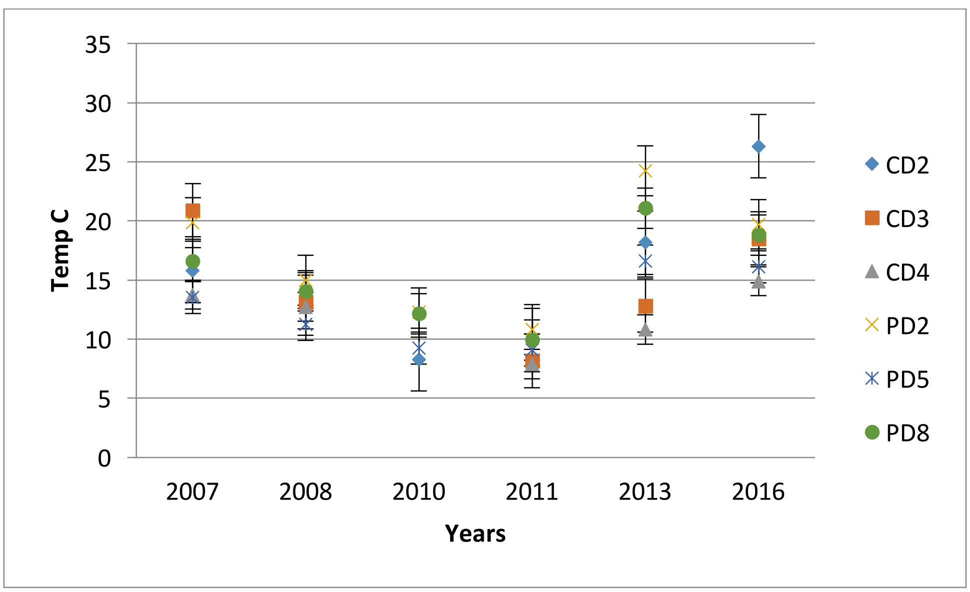

Figure 2: Mean temperatures (±SE) measured during biological monitoring from 2007-2016.

Dissolved oxygen concentrations were above the recommended 6 mg/L for warm water fish (Davidson and Rule 2006) at most sites during most years (Figure 3). However, DO levels were below this value at most sites during 2007, and at CD2 and CD4 during other years. Dissolved oxygen concentrations tended to be highest in the permanently-flowing, groundwater influenced sites in Pine Draw (PD5 and PD8), and lower in heavily impacted Company Ditch sites (particularly CD2 and CD4). DO concentrations were consistently high in 2011, when water temperatures were consistently cold, and more variable in years when temperature was more variable; the solubility of oxygen decreases as water temperature increases.

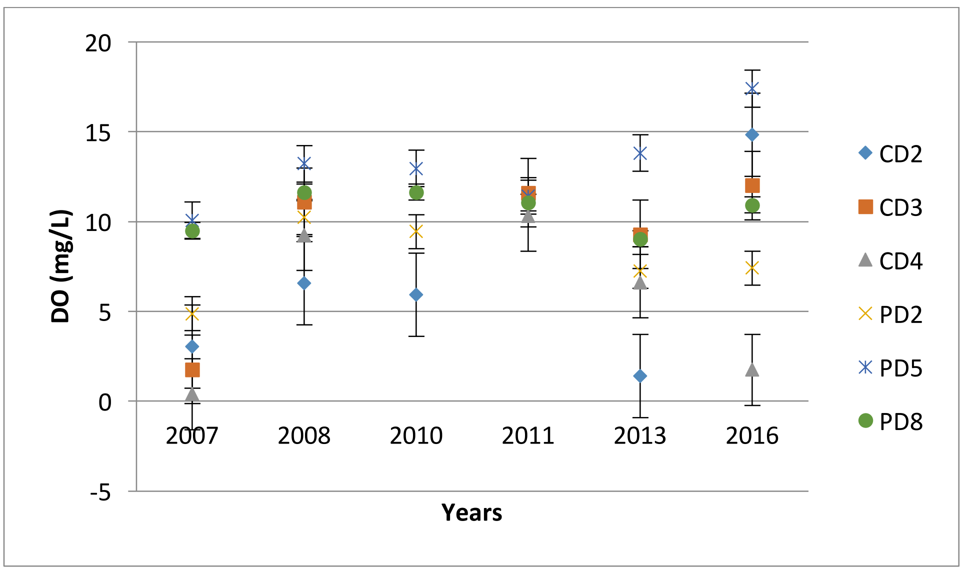

Figure 3: Mean DO (±SE) measured during biological monitoring from 2007-2016.

Water at most sites and dates was slightly basic (Fig. 4), with measured pH ranging from 6.9 (CD4, 2011) to 9.5 (PD8, 2010).   Overall, pH was higher (more basic) in the permanent Pine Draw sites (PD5 and PD8) compared to season sites. Highest pH was observed in PD8.

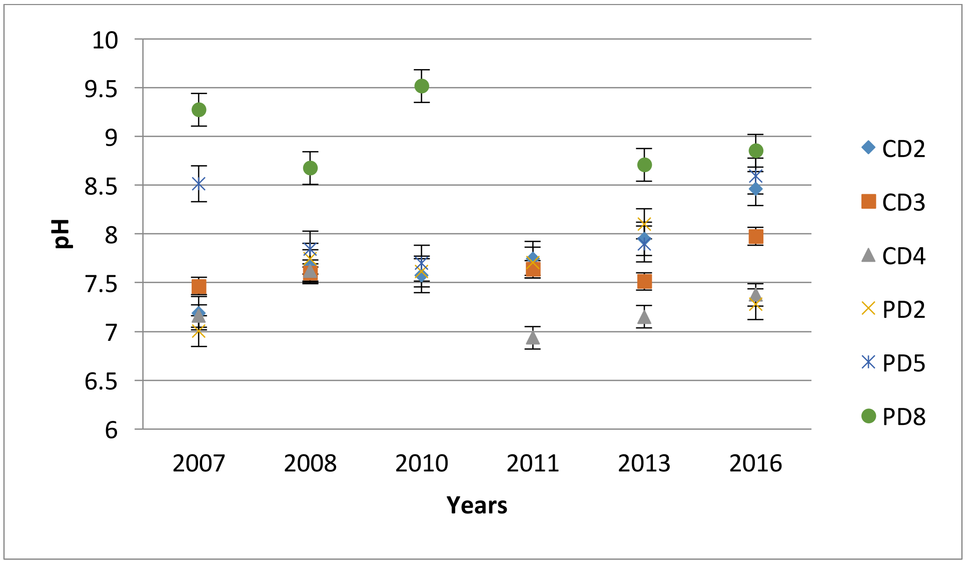

Figure 4: Mean pH (±SE) measured during biological monitoring from 2007-2016.

Conductivity was generally lower in the permanent Pine Draw sites (PD5 and PD8) compared to seasonal sites (Fig. 5). The highest mean conductivity observed was CD2 during 2013 at 1.815 mS. Higher conductivity generally indicates greater effects of evaporation or more inputs from overland flow, and can also indicate septic influence. In Pine Draw, lower conductivity is associated with groundwater inputs, which may explain why conductivity was typically lowest at PD8.

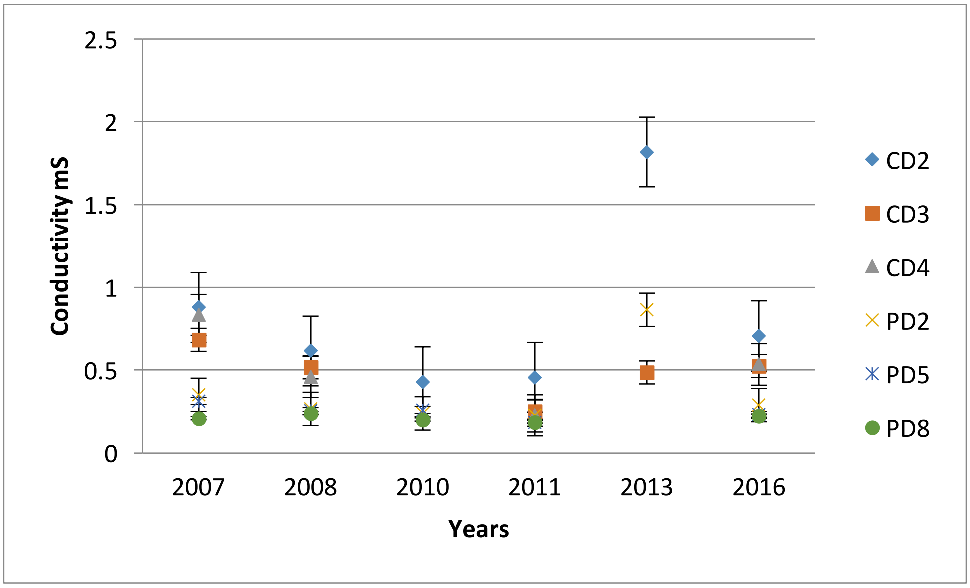

Figure 5: Mean conductivity (±SE) measured during biological monitoring 2007-2016.

Channel width for the sites sampled averaged about 4 m in most years (Figure 6), with mean depth ranging from 10 to 45 cm (Figure 7). The channel was widest, deepest, and most variable between years at PD8, the most downstream site within Pine Draw.

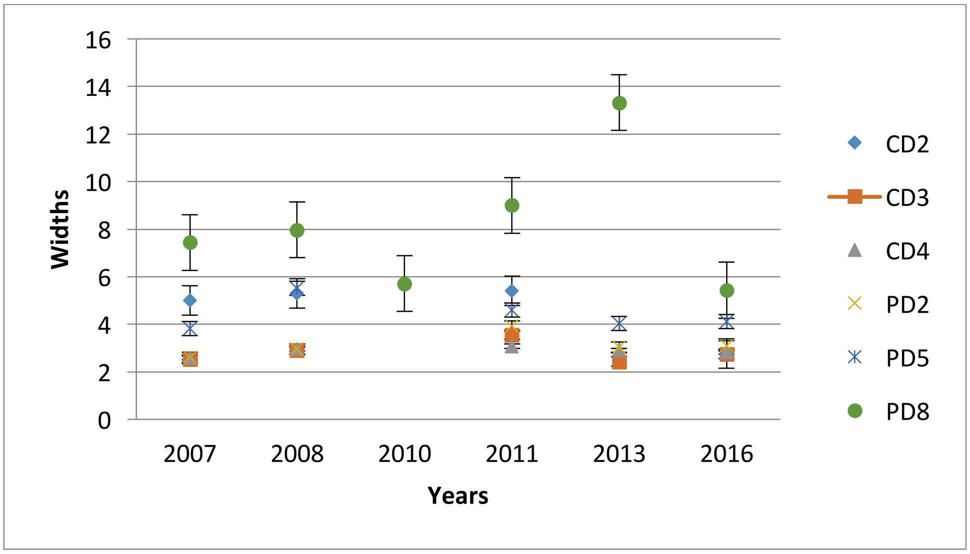

Figure 6: Mean widths (±SE) measured during biological monitoring 2007-2016.

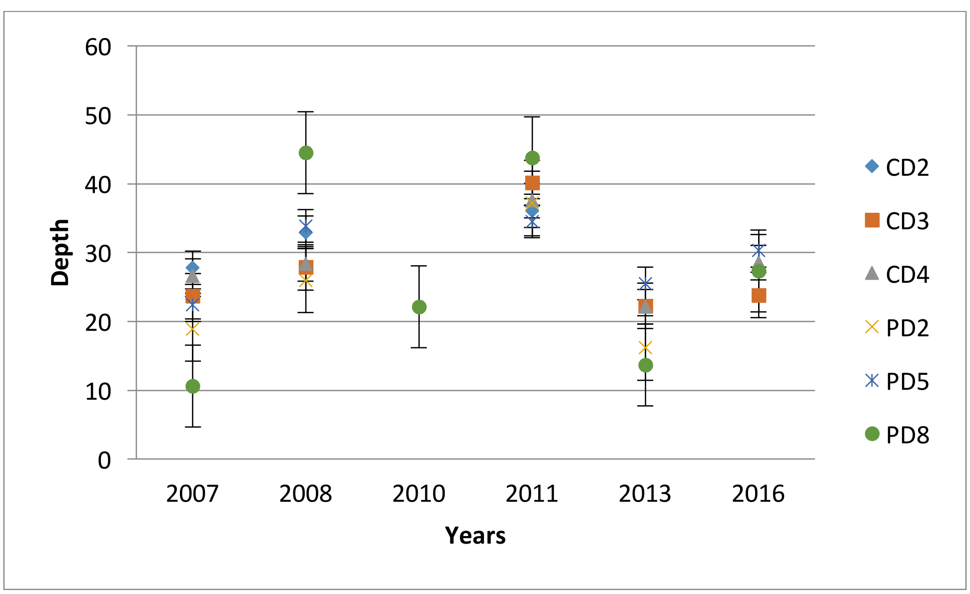

Figure 7: Mean depths (±SE) measured during biological monitoring 2007-2016

Data for dissolved nutrients are incomplete prior to 2016. However, soluble reactive phosphorus data were available for all sites and years except 2011. Phosphate levels have been consistently high in Company Ditch, but may be declining towards the end of the study period. Nitrate levels were slightly high, but not near EPA recommended limits, in PD5 from 2011 through 2016.

Table 2: Soluble reactive phosphorus (SRP) and inorganic nitrogen measurements (NH_4^+-N, NO_3^-N), 2008-2016.

Site	Year	NH4+ N ppb	NO3- N ppb	SRP ppb
CD2	2008	39		637
CD3	2008	3		359
CD4	2008	6		107
PD2	2008	10		16
PD5	2008	17		58
PD8	2008	36		11
CD2	2010	8		675
CD3	2010	7		656
CD4	2010	32		243
PD2	2010	20		14
PD5	2010	23		83
PD8	2010	7		4
CD2	2011		< 5	
CD3	2011		< 5	

CD4	2011			
PD2	2011		858	
PD5	2011		1370	
PD8	2011		462	
CD2	2013	60	< 5	629
CD3	2013	25	41	336
CD4	2013	21		87
PD2	2013	11	< 5	32
PD5	2013	38	2805	97
PD8	2013	4	96	31
CD2	2016	18	9	228
CD3	2016	25	14	250
CD4	2016	20	15	102
PD2	2016	61	14	11
PD5	2016	11	1420	24
PD8	2016	50	206	5

Comparison of Spring vs. Fall 2016 within Pine Draw

Three sites within Pine Draw remained wetted through September 2016. The remaining 7 sites sampled in spring 2016 were dry by early September 2016. Among the 3 sites resampled in fall, water temperatures varied significantly with site, season, and site by season interaction (Table 3). Mean water temperatures were lower during fall (September) compared to Spring (May) (Fig. 8), opposite of variation in air temperature, which was warmer in early fall. The colder water temperatures later in the year likely reflect increased contributions of groundwater compared to surface water to the flow of the sites as the season became drier (McNeely and Nezat, unpublished data). The temperatures of the sites were more similar to each other during the spring season and they showed differences from each other during the fall season. The highest temperature was measured at PD8 during the spring - 18.8 (SD 0.06), and the lowest temperature was measured at PD3 during the fall 6.8 (SD 0.54).

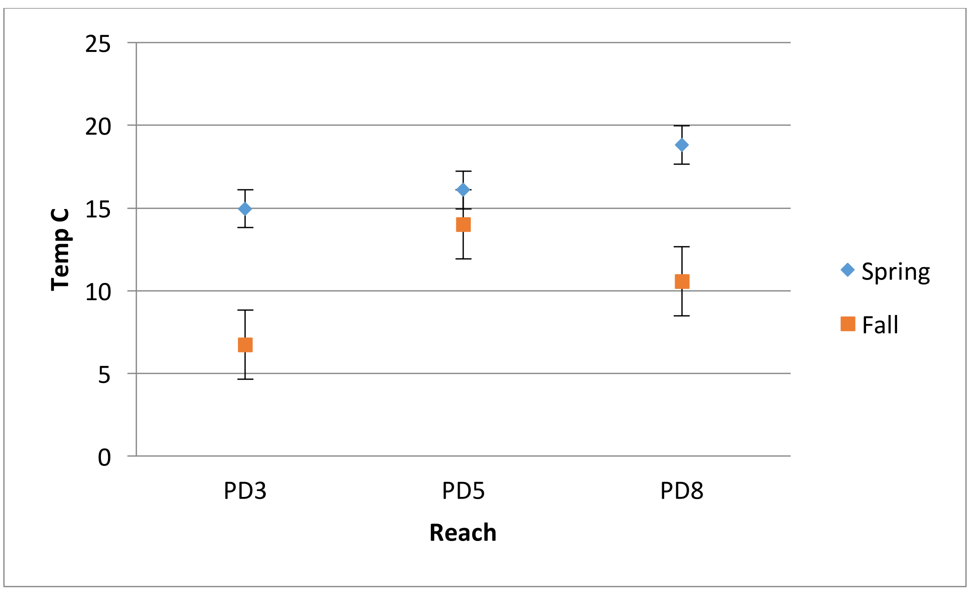

Figure 8: Mean temperatures (±SE) at Pine Draw sites in Spring (May) and Fall (September) 2016.

Table 3. Two-way ANOVA of water temperature with independent variables site and season for Pine Draw sites in Spring (May) and Fall (September) 2016.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Site	573	1	573	4061	<0.0001
Season	215	2	107	761	<0.0001
Interaction	123	2	62	437	<0.0001
Residual	7.6	54	0.14		
Total	919	59			

Mean dissolved oxygen concentrations (DO) varied significantly with site, season, and site by season interaction. DO was lower during the season of fall, whereas during the spring season the DO remained relatively higher, especially that of PD5 site (Fig. 9). Moreover, the DOs of different PD sites remained closer to each other during the fall season and they showed differences from each other during the spring season. The highest value was obtained from PD5 site during the spring season, when the DO was 17.4 mg/L. The lowest value was obtained from the PD3 site during the fall season, when the DO was 5.9 mg/L.

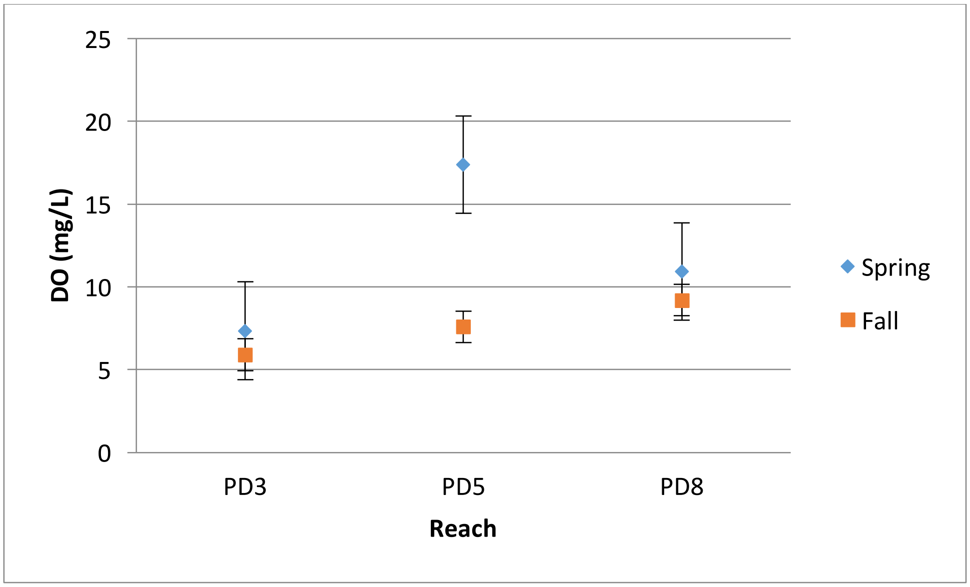

Figure 9: Mean DO (±SE) at Pine Draw sites in Spring (May) and Fall (September) 2016.

Table 4. Two-way ANOVA of dissolved oxygen concentration with independent variables site and season for Pine Draw sites in Spring (May) and Fall (September) 2016.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Site	279	1	279	284	<0.0001
Date	357	2	179	182	<0.0001
Interaction	215	2	107	110	<0.0001
Residuals	53	54			
Total	904	59			

There was significant variation in pH of the Pine Draw with site, date, and site by date interaction (Fig. 10, Table 5). Mean pH was lower during the season of fall, whereas during the spring season the pH remained relatively higher, especially that of PD8 site. Moreover, the pH of different PD sites remained closer to each other during the fall season and they showed differences from each other during the spring season. The highest value was obtained from PD8 site during the spring season (8.9); the lowest value was obtained from the PD8 site during the fall season (6.8)

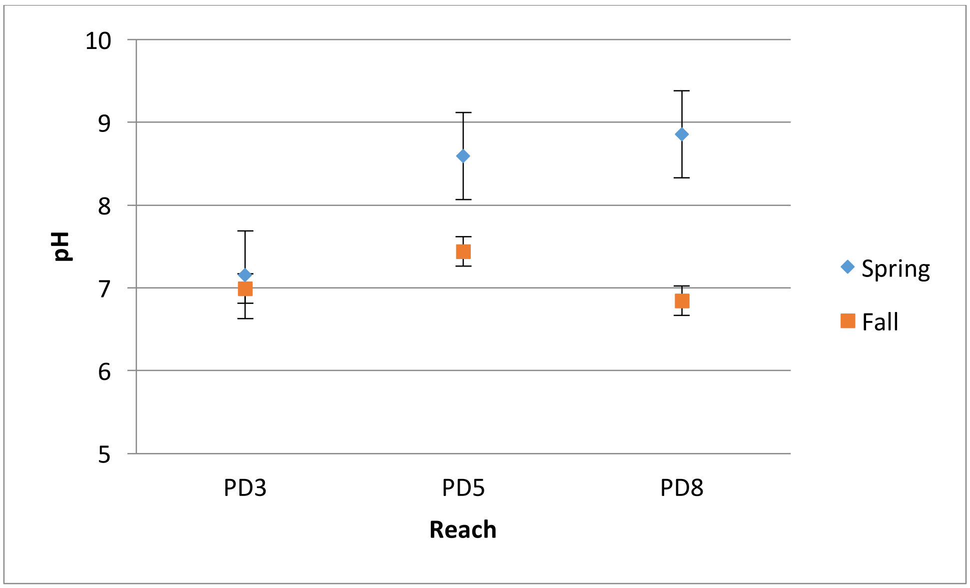

Figure 10: Mean pH (±SE) at Pine Draw sites in Spring (May) and Fall (September) 2016.

Table 5. Two-way ANOVA of pH with independent variables site and season for Pine Draw sites in Spring (May) and Fall (September) 2016.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P*
Site	18.6	1	18.6	1220	<0.0001
Season	10.1	2	5.05	332	<0.0001
Interaction	8.64	2	4.32	284	<0.0001
Residual	0.821	54	0.0152		
Total	38.1	59			

Mean conductivity varied with site, season, and site by season interactions (Table 6. Fig. 11). The conductivity measurements of different PD sites remained closer to each other during the spring and they showed differences from each other during the fall (Fig. 11). PD8 had the lowest value site during the fall, when the conductivity was 0.141 mS. This low value was likely the result of groundwater inputs.

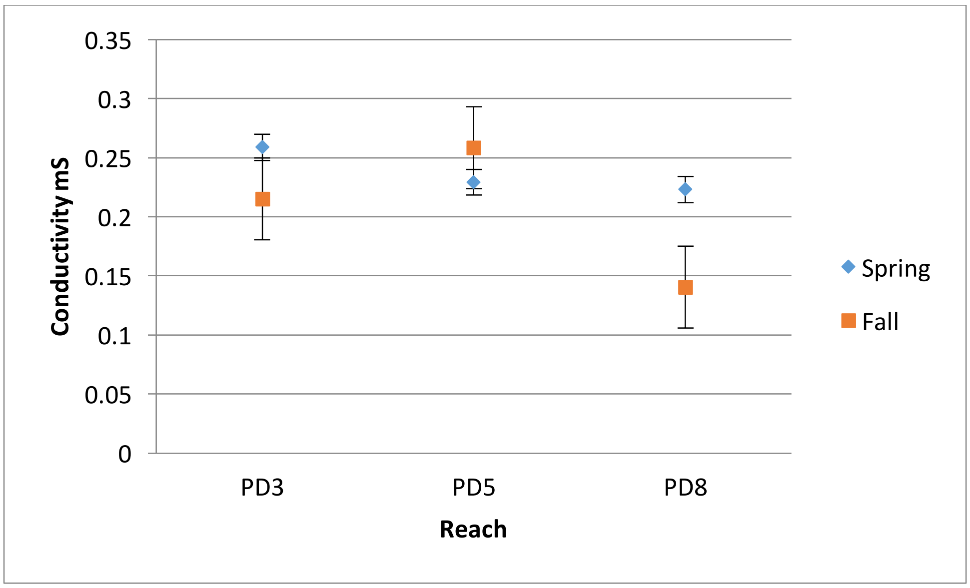

Figure 11: Mean conductivity (±SE) at Pine Draw sites in Spring (May) and Fall (September) 2016.

Table 6. Two-way ANOVA of conductivity with independent variables site and season for Pine Draw sites in Spring (May) and Fall (September) 2016.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Site	0.0157	1	0.0157	311	<0.0001
Season	0.0463	2	0.0231	460	<0.0001
Interaction	0.0321	2	0.0160	319	<0.0001

Residual	0.00272	54	5.03E-05
Total	0.0968	59	

During spring 2016 sampling, nitrogen and phosphorous concentrations were relatively low at all Pine Draw stream sites (Table 7). In fall, ammonium levels were high at PD5 and PD8, perhaps as a result of decomposition releasing ammonium in nearby wetlands. Soluble reactive phosphorous levels were slightly elevated at PD5 in fall.

Table 7. Soluble reactive phosphorus (SRP) and Nitrogen measurements (NH_4^+-N, NO_3^--N), for Pine Draw sites sampled in spring and fall 2016.

Season	Site	Date	NH4+ N ppb	NO3- N ppb	SRP ppb
Fall	**PD3**	7-Sep-16	21	< 5	24
Fall	**PD5**	7-Sep-16	506	844	78
Fall	**PD8**	7-Sep-16	138	672	36
Spring	**PD3**	12-May-16	28	16	15
Spring	**PD5**	3-May-16	11	1420	24
Spring	**PD8**	3-May-16	50	206	5.3

Spring 2016 Analysis, 10 sites

Water temperatures ranged from 13.1 (CT1) to 26.3 (CD2) when sites were sampled in spring 2016 (Fig. 12), a very broad range in terms of tolerances of aquatic organisms. Water temperature decreased downstream in Company Ditch from CD2 to CD4, and increased downstream in Pine Draw from PD3 to PD8. Among the additional sites from other watersheds, CT1 was colder than Company Ditch and Pine Draw sites, while KD3 and PH3 were comparable to most Company Ditch and Pine Draw sites.

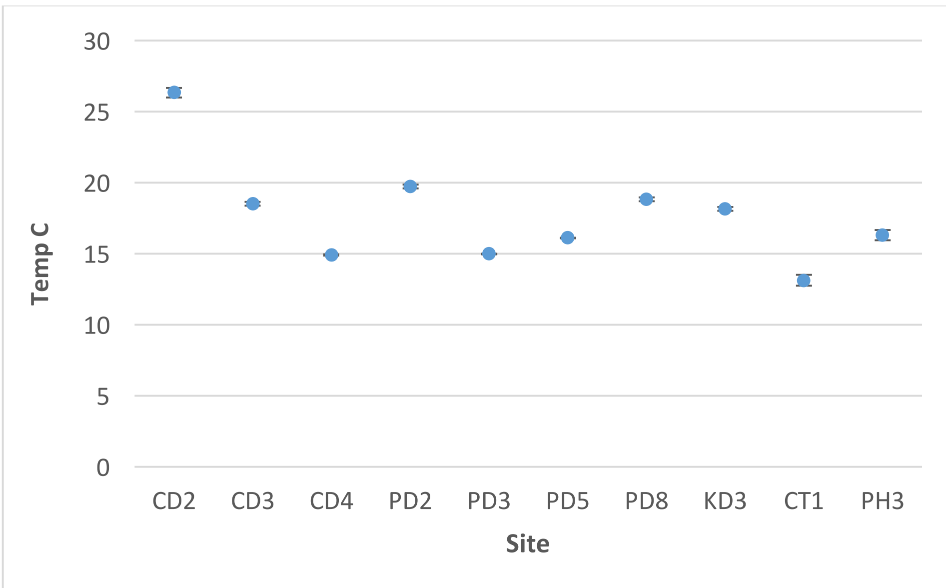

Figure 12. Mean water temperature (±SE) for 10 sites on TNWR, spring 2016.

Dissolved oxygen concentrations (DO) ranged from hypoxic (CD4 and CT1) to supersaturated (CD2, CD3 and PD5, Fig. 13). Supersaturated DO conditions usually result from high primary production, and may indicate high nutrient conditions. Low dissolved oxygen concentrations at CD4 and CT1 could be stressful for aquatic life.

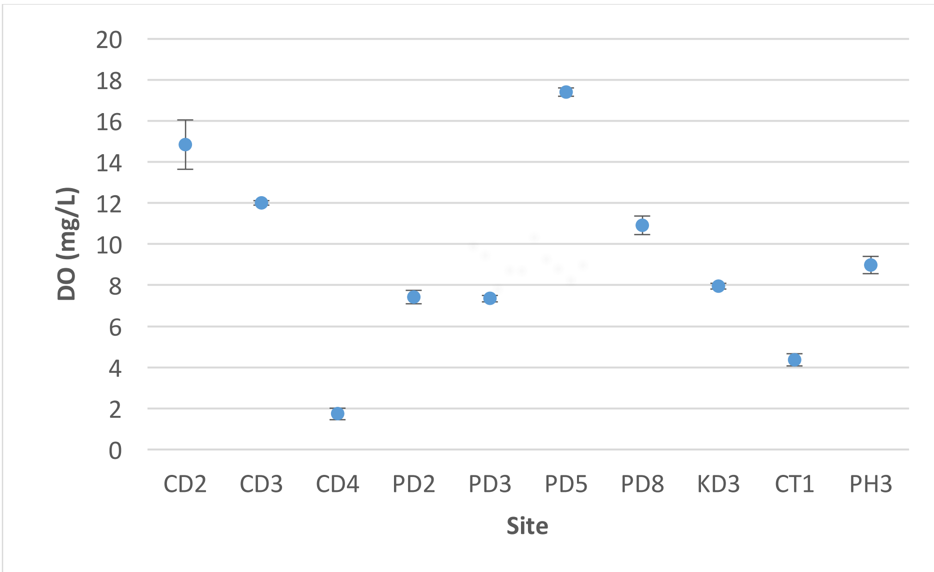

Figure 13. Mean dissolved oxygen concentrations (±SE) for 10 sites on TNWR, spring 2016.

Water was slightly basic across all sites sampled in spring 2016, with pH ranging from 7.2 (PD3) to 8.9 (PD8, Fig. 14). pH was highest in the most downstream Pine Draw sites (PD5 and PD8). The three sites from adjacent watersheds (CT1, KD3, and PH3) were comparable to the other Pine Draw and Company Ditch sites.

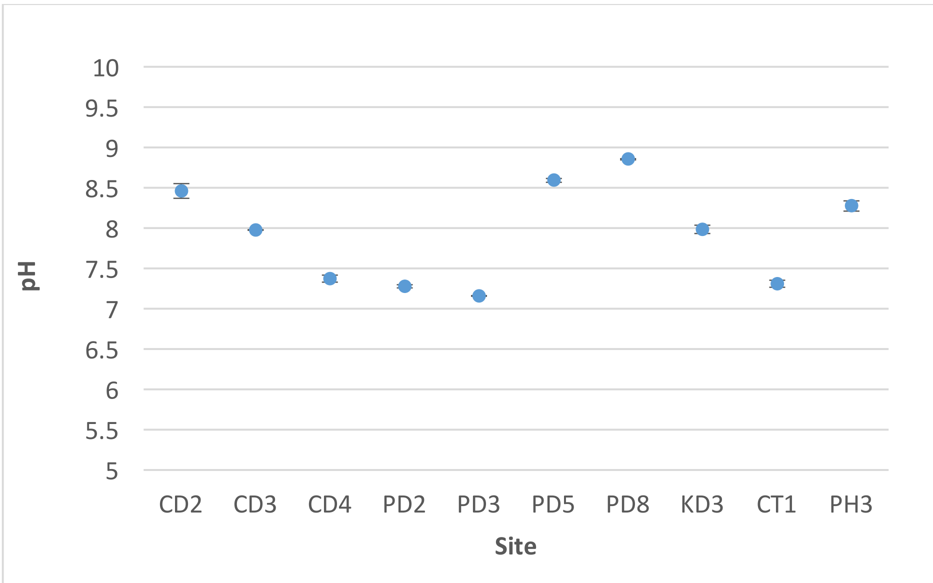

Figure 14. Mean pH (±SE) for 10 sites on TNWR, spring 2016.

Conductivity measurements were high in CD2 and KD3, indicating substantial effects of evaporation, runoff, or possible septic influence (Fig. 15). CD3 and CD4 had higher conductivity than most sites, but lower than CD2, suggesting inputs of water from another source. Pine Draw sites had lower conductivity than sites from other watersheds.

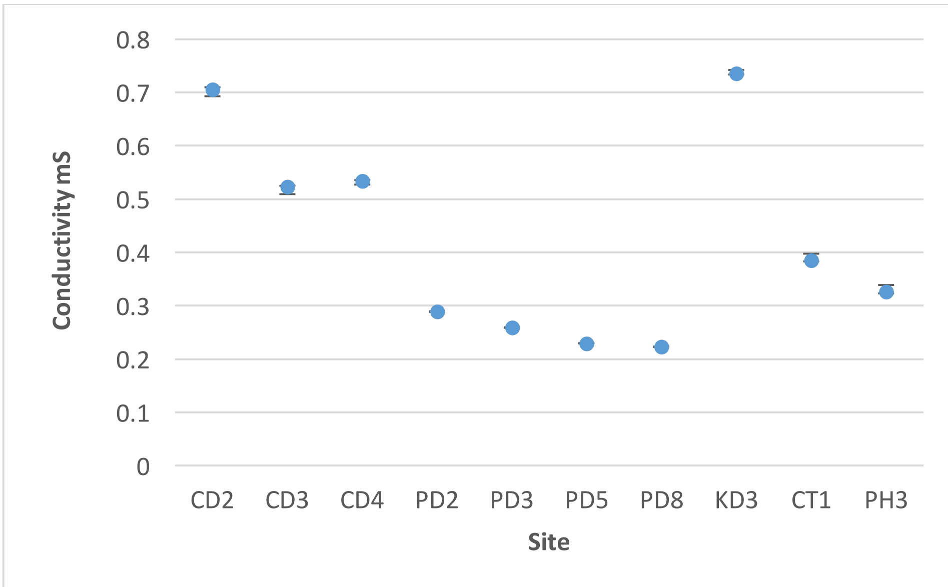

Figure 15. Mean pH (±SE) for 10 sites on TNWR, spring 2016.

During spring 2016 sampling, nitrogen and phosphorous concentrations were relatively low at most stream sites (Table 7). However, soluble reactive phosphorous remained elevated at all the Company Ditch sites, and were slightly elevated in Philleo Ditch (PH3). Nitrogen levels were elevated in Philleo Ditch (PH3), particularly for ammonium.

Table 8: Soluble reactive phosphorus (SRP) and Nitrogen measurements (NH_4^+-N, NO_3^--N), spring 2016.

Site	Date	NH4+ N ppb	NO3- N ppb	SRP ppb
CD2	3-May-16	18	9	228
CD3	26-Apr-16	25	14	250
CD4	26-Apr-16	20	15	102
KD3	26-May-16	26	< 5	23
CT1	26-May-16	45	15	17
PH3	19-May-16	190	3050	86
PD2	3-May-16	61	14	11
PD3	12-May-16	28	16	15
PD5	3-May-16	11	1420	24
PD8	3-May-16	50	206	5.3

Macroinvertebrate Species

Annual Comparisons, 2007-2016

The Hilsenhoff Family Biotic Index (HFBI) varied significantly among sites, among years, and with a year by site interaction (Figures 16 & 17, Table 8). Lower HFBI values indicate lower levels of pollution and higher water quality (Table 1). Over all sites, HFBI values were lowest (highest water quality) in 2007, and highest (poorest water quality) in 2008 and 2016. Averaging over all years, Pine Draw site PD2 and Company Ditch site CD3 had significantly lower HFBI compared to all other sites, which were not significantly different from each other (Fig. 17). According to Hilsenhoff (1988), the lowest values observed (Pine Draw site PD2 prior to 2013, and Company Ditch site PD3 after 2010) correspond to "very good" water quality (Fig. 18). The highest values (Company Ditch sites 2 and 4, Pine Draw site 8 in 2016) observed correspond to "poor" or "very poor" water quality. Company Ditch site 3 improved dramatically from 2008-2010, however, we did not observe similar improvement at Company Ditch site 4. Scores for Pine Draw site 2 increased (indicating poorer water quality) from 2011 to 2016.

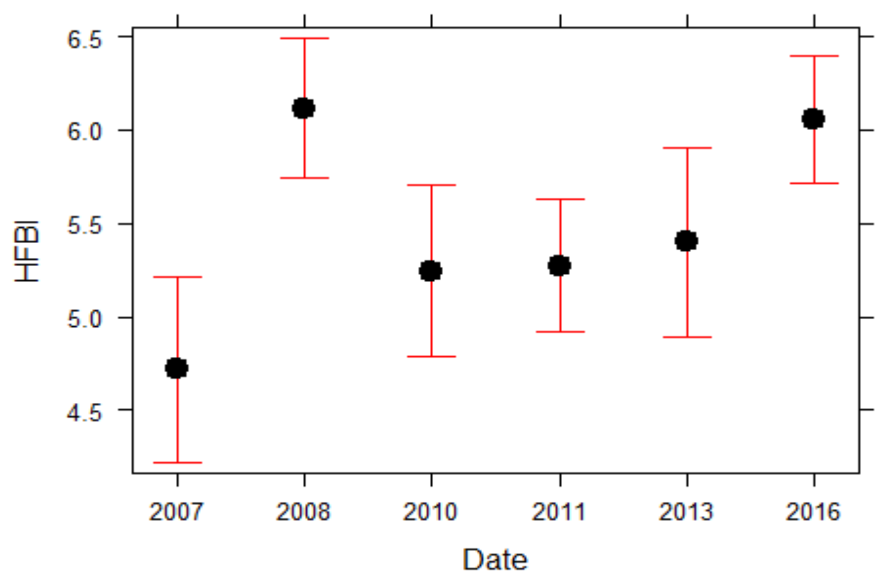

Figure 16. Mean Hilsenhoff Family Biotic Index values (with 95% confidence intervals) across all sites for years 2007-2016.

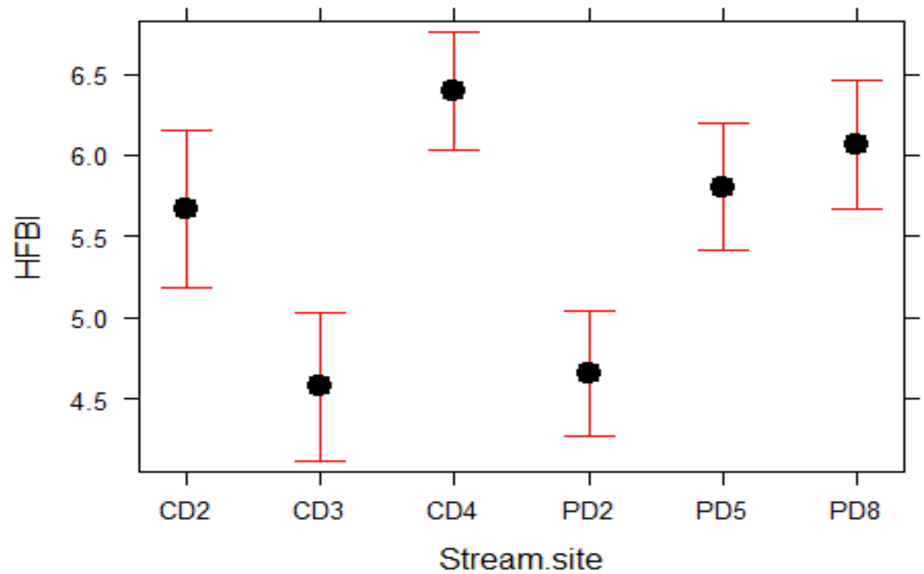

Figure 17. Mean Hilsenhoff Family Biotic Index values (with 95% confidence intervals) across all years for biological monitoring sites.

Table 9. Two-way ANOVA of HFBI scores for biological monitoring sites from 2007 to 2016.

	Df	SS	MS	F value	Pr(>F)
Year	5	29.75	5.949	6.198	<0.0001
Site	5	64.09	12.818	13.353	<0.0001
Year x Site	23	64.76	2.816	2.933	<0.0001
Residuals	117	112.31	0.960		

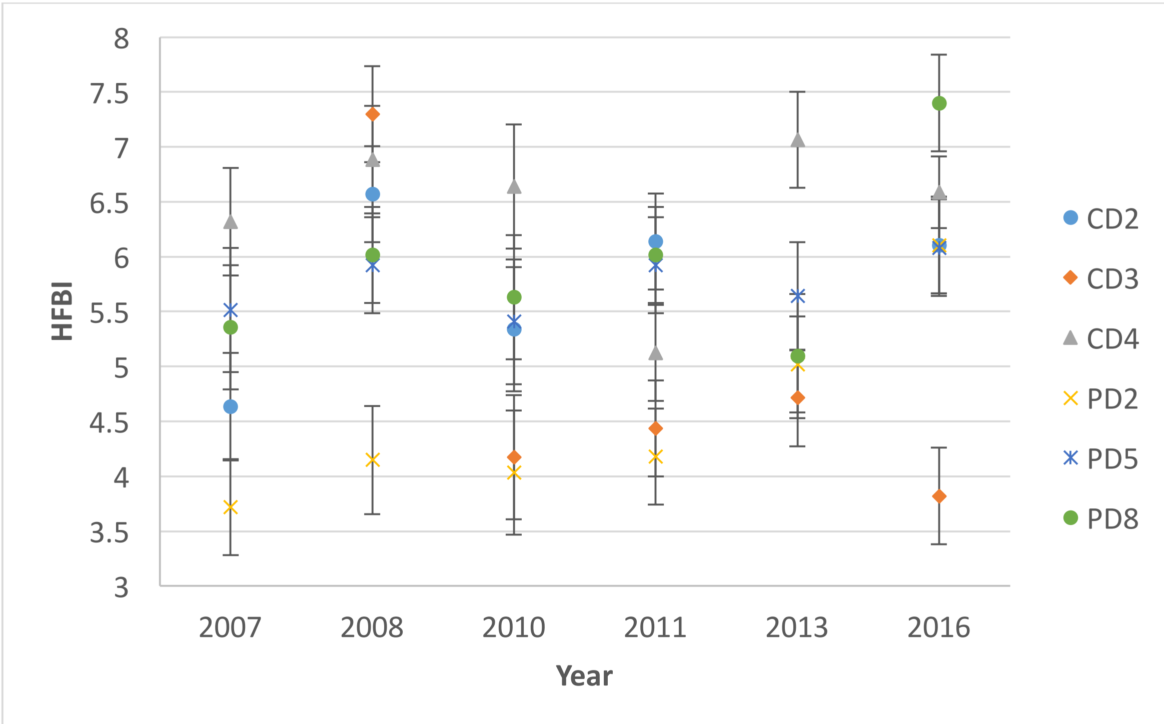

Figure 18. Mean Hilsenhoff Family Biotic Index scores (± S.E.) for Company Ditch and Pine Draw sites, 2007-2016.

The proportion of the community comprised of the dominant taxon varied significantly by site, year, and with a site by year interaction (Table 9, Figs 19 & 20). Domination by a single taxon usually indicates poor water quality or disturbed ecological conditions. Overall, sites were most dominated by single taxa in 2008 – this year differed significantly from all other years. In most years, Company Ditch sites were more dominated by a single taxon than Pine Draw sites. Over all years, CD4 had a significantly higher proportion dominant taxon compared to other sites and PD5 and PD8 had a significantly lower proportion dominant taxon. CD2, CD4, and CD3 prior to 2010 were highly dominated by Ostracods (seed shrimp), a small crustacean with a short life cycle that is very tolerant of poor water quality and habitat conditions. Ostracods have become much less dominant and the community more diverse in Company Ditch over time, indicating increasing water quality. Pine Draw sites were often dominated by Gammaridae (freshwater amphipods) and Chironomidae (midge larvae) throughout the years 2007 to 2013. Gammaridae and Chironomidae may be common in a wide variety of water quality conditions. Beginning in 2010, CD3 has been dominated by mayflies in the families Leptophlebiidae or Baetidae. These mayflies have been very abundant in PD2, including dominant in some years, and may be indicators of good water quality and habitat conditions for seasonal streams in this area. Reduced domination of Company Ditch sites by Ostracods may indicate improving water quality and ecosystem integrity during 2008-2011 following the closing of the dairy.

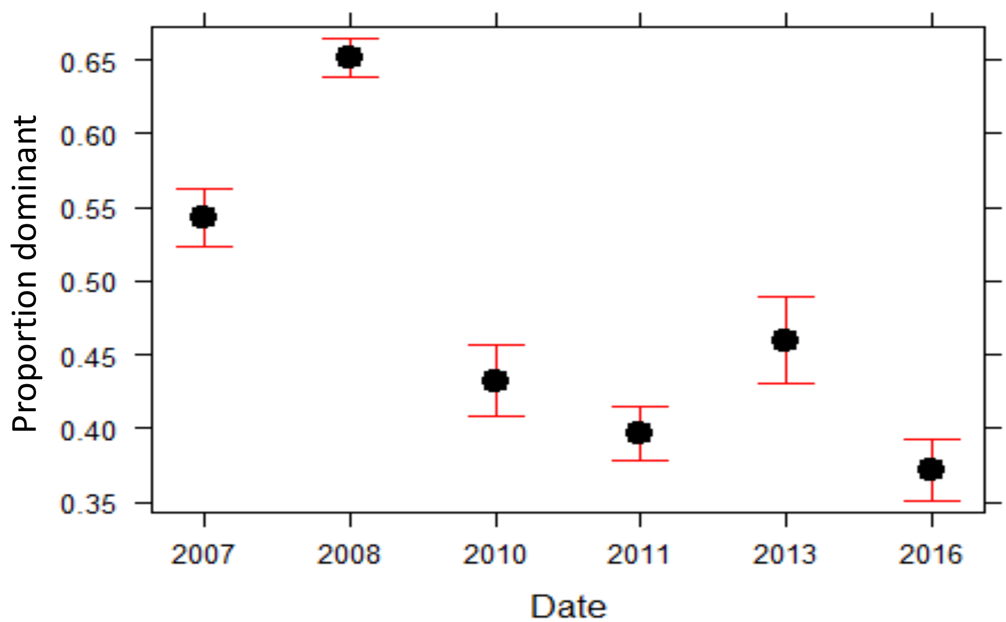

Figure 19. Mean proportion of individuals that consisted of the single dominant taxon (with 95% confidence intervals) across all sites for years 2007-2016.

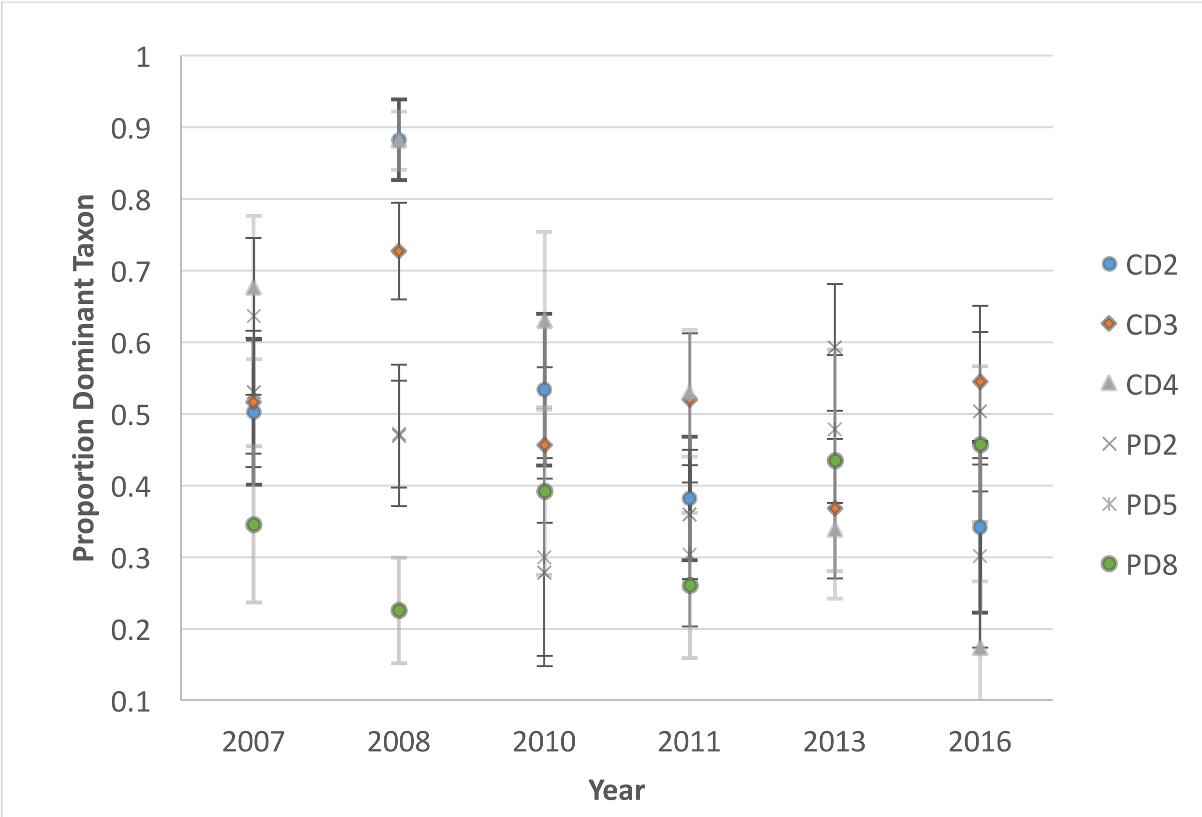

Figure 20. Mean (± SE) proportion of individuals made up of each site's dominant taxon for biological monitoring sites, 2007-2016.

Table 10. Logistic regression results for frequency of the dominant taxon among individuals collected from biological monitoring sites, 2007 to 2016.

	DF	Residual Dev	Dev	P
Null	156	9507		
Site	5	2498	7008	<0.0001
Year	5	2087	4925	<0.0001
Site x Year	24	1874	3051	<0.0001

The proportion of the community with a multivoltine life history varied significantly by site, year, and with a site by year interaction (Table 10, Figs 21 & 22). Most of the individuals collected were multivoltine at all sites and years with the exception of PD2 in 2007, which was dominated that year by univoltine Leptophlebiid mayflies. Over all sites, proportion multivoltine was highest in 2008 (nearly 1) and lowest in 2011 (0.77).

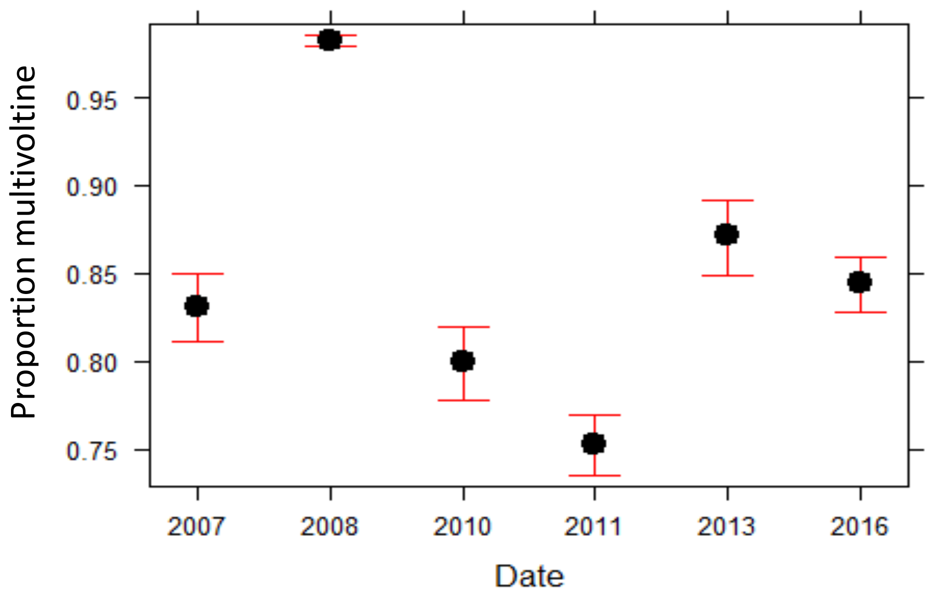

Figure 21. Mean proportions of individuals with a multivoltine life history (with 95% confidence intervals) across all sites for years 2007-2016.

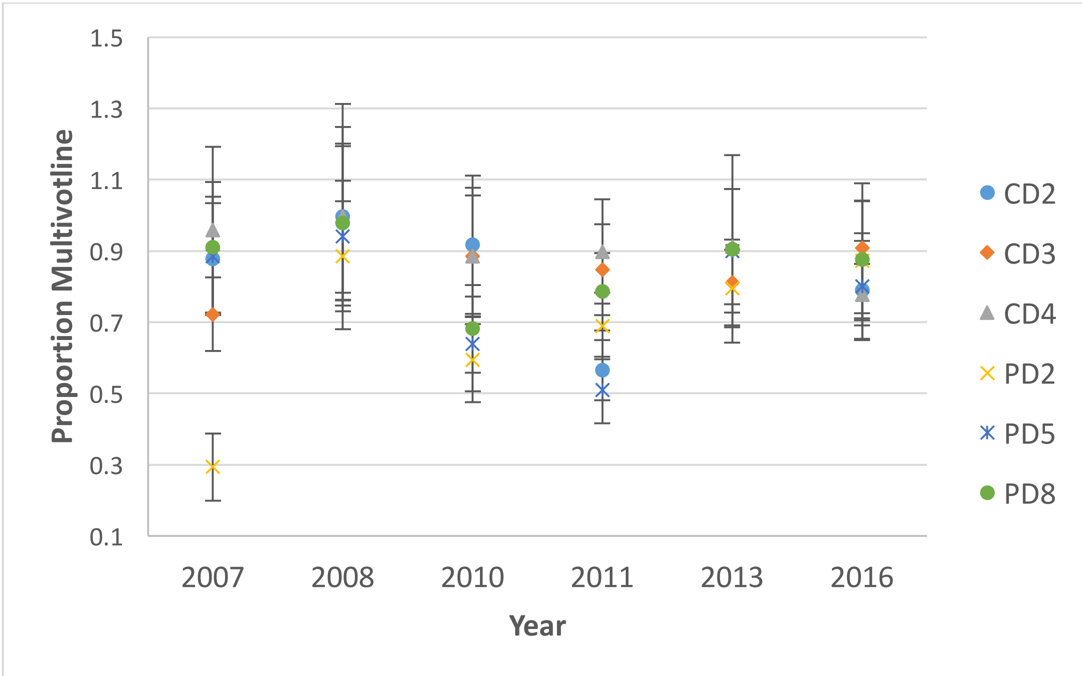

Figure 22. Mean (± SE) proportion of individuals with a multivoltine life history for biological monitoring sites, 2007-2016.

Table 11. Logistic regression results for frequency of multivoltine life history among individuals collected from biological monitoring sites, 2007 to 2016.

	DF	Residual Dev	Dev	P
Null	156	6075		
Site	5	1616	4459	<0.0001
Year	5	2155	2304	<0.0001
Site x Year	24	1076	1228	<0.0001

The proportion of the community with a wetland habitat affinity varied significantly by site, year, and with a site by year interaction (Table 11, Figs 23 & 24). Permanent Pine Draw sites (PD5 and PD8) typically had low proportion of wetland species. The proportion of wetland taxa in seasonal sites (Company Ditch sites and PD2) was higher and more variable from year to year. The proportion of wetland taxa declined in Company Ditch sites from 2010 to 2011, and remained lower in these sites. Wetland taxa are not specifically indicators of water quality, but may be indicators of strong linkages with adjacent wetlands or hydrological changes over time.

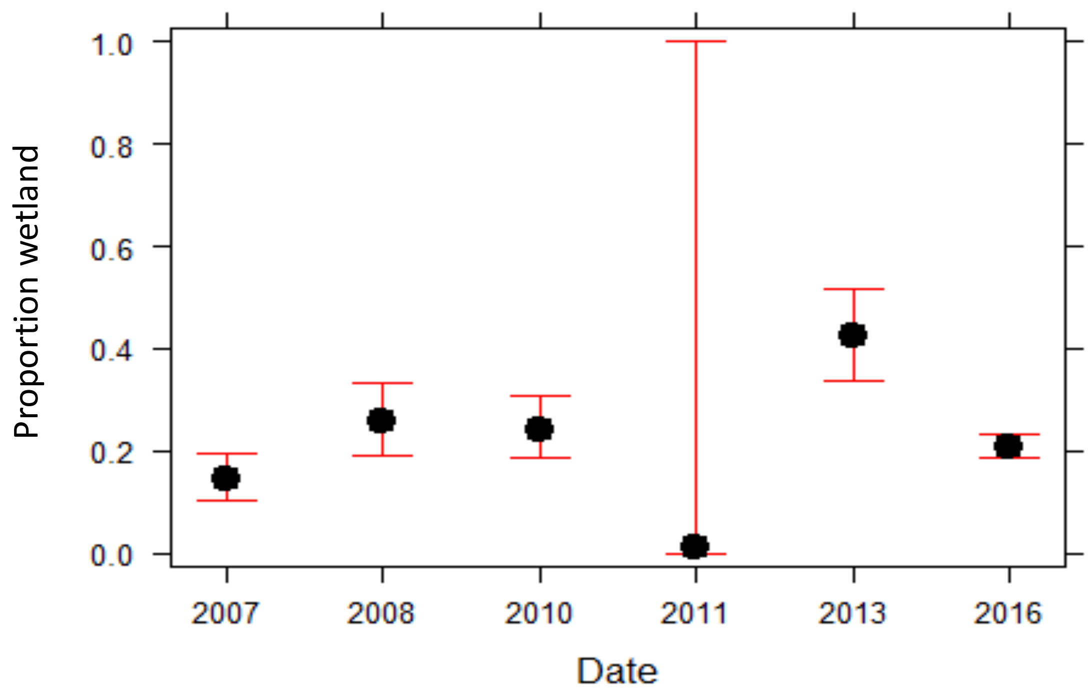

Figure 23. Mean proportions of individuals with wetland habitat affinity (with 95% confidence intervals) across all sites for years 2007-2016. The large errors in the 2011 estimate were due to one site (PD5) having no individuals with wetland affinity sampled that year.

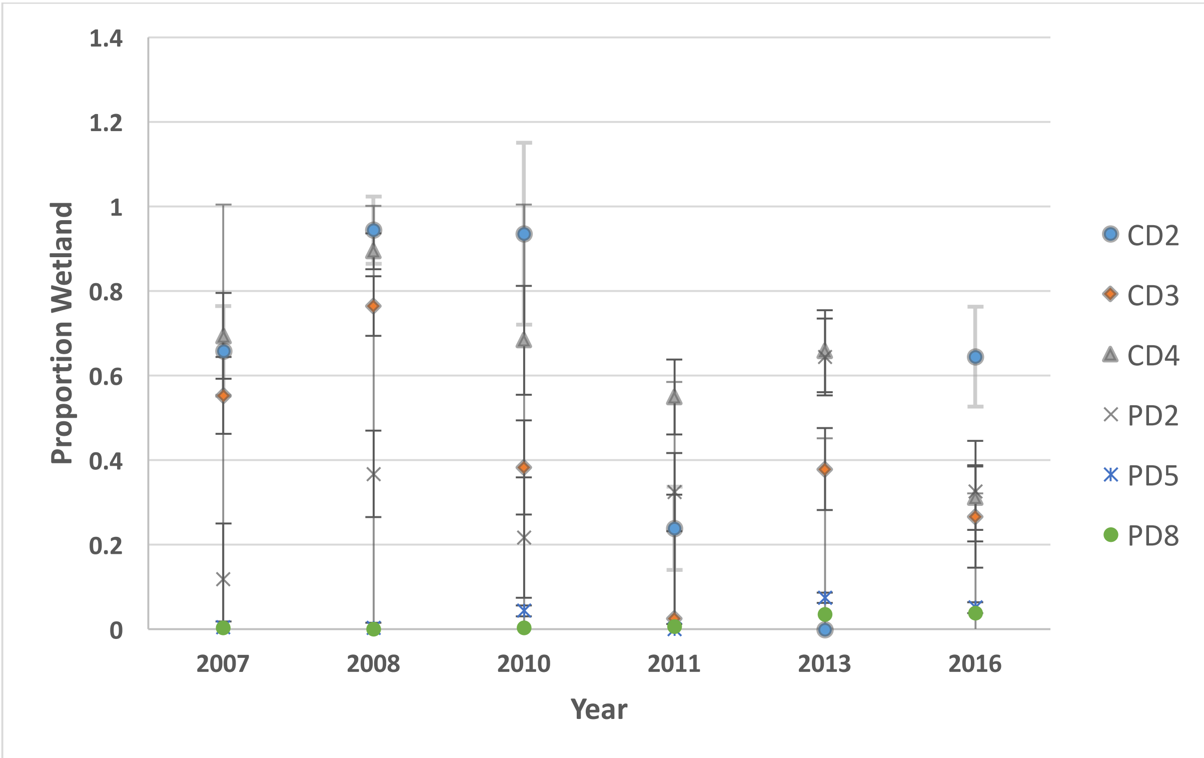

Figure 24. Mean (± SE) proportion of individuals with wetland habitat affinity for biological monitoring sites, 2007-2016.

Table 12. Logistic regression results for frequency of wetland habitat affinity among individuals collected from biological monitoring sites, 2007 to 2016.

	DF	Residual Dev	Dev	P
Null	156		18521	
Site	5	11406	7115	<0.0001
Year	5	3063	4051	<0.0001
Site x Year	24	1416	2635	<0.0001

Comparison of Spring vs. Fall 2016

HFBI scores varied significantly with season (spring vs. fall), and the interaction between season and site, but not with site alone (Table 12, Fig. 24). In this case, the fall and spring data seem to be a complete inversion of each other in regard to the quality of water. In fall, PD3 and PD8 had lower values of HFBI than PD5. In spring, values were high in PD3 and PD8, and lower in PD5.

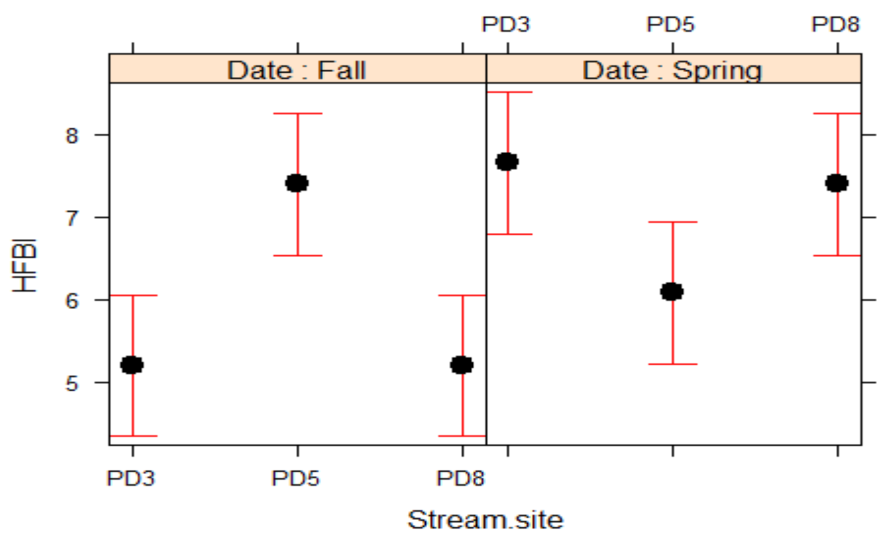

Figure 25. Mean HFBI scores for macroinvertebrate communities sampled in spring (April/May) and fall (September) from 3 sites in Pine Draw, TNWR.

Table 13. Two-way ANOVA for HFBI of 3 sites in Pine Draw, TNWR, with site and season as independent variables.

	Df	SS	MS	F value	Pr(>F)
Site	2	1.027	0.514	0.595	0.560
Season	1	9.307	9.307	10.777	0.003
Site x season	2	22.26	11.132	12.890	< 0.001
Residuals	24	20.73	0.864		

The proportion of the community comprised of the dominant taxon varied significantly by site, season, and with a site by season interaction (Table 13, Fig. 26). As with HFBI, data on the proportion the dominant taxon suggested different patterns of water quality among the sites in spring and fall. Overall, Pine Draw macroinvertebrates were more diverse and less dominated by a single taxon in fall compared to spring. In spring, PD8 had the highest proportion of the dominant taxon and PD5 the lowest. In fall, PD5 and PD8 were similar, and less dominated by a single taxon than PD3. The dominant taxa present at all three sites were different between fall and spring, indicating seasonal turnover.

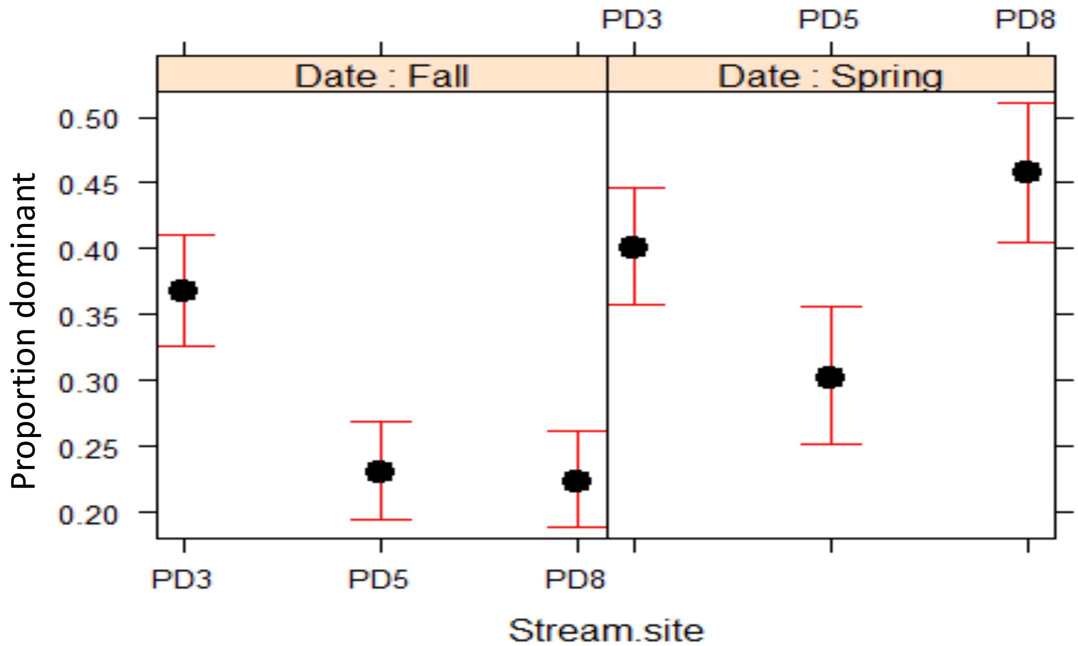

Figure 26. Mean proportions of the community comprised on the dominant taxon for macroinvertebrate communities sampled in spring (April/May) and fall (September) 2016 from 3 sites in Pine Draw, TNWR.

Table 14. Logistic regression results for frequency of the single dominant taxon among individuals collected from 3 sites on Pine Draw, spring and fall 2016.

	DF	Residual Dev	Dev	P
Null	29	600		
Site	2	33	567	<0.0001
Season	1	34	532	<0.0001
Site x Season	2	26	510	<0.0001

The proportion of the individuals with a multivoltine life history varied significantly by site, season, and with a site by season interaction (Table 14, Fig. 27). The multivoltine life history strategy was dominant in Pine Draw during spring 2016, and much less dominant in fall 2016. In fall, but not spring, multivoltine life history strategies were less dominant in PD3 compared to PD5 and PD8.

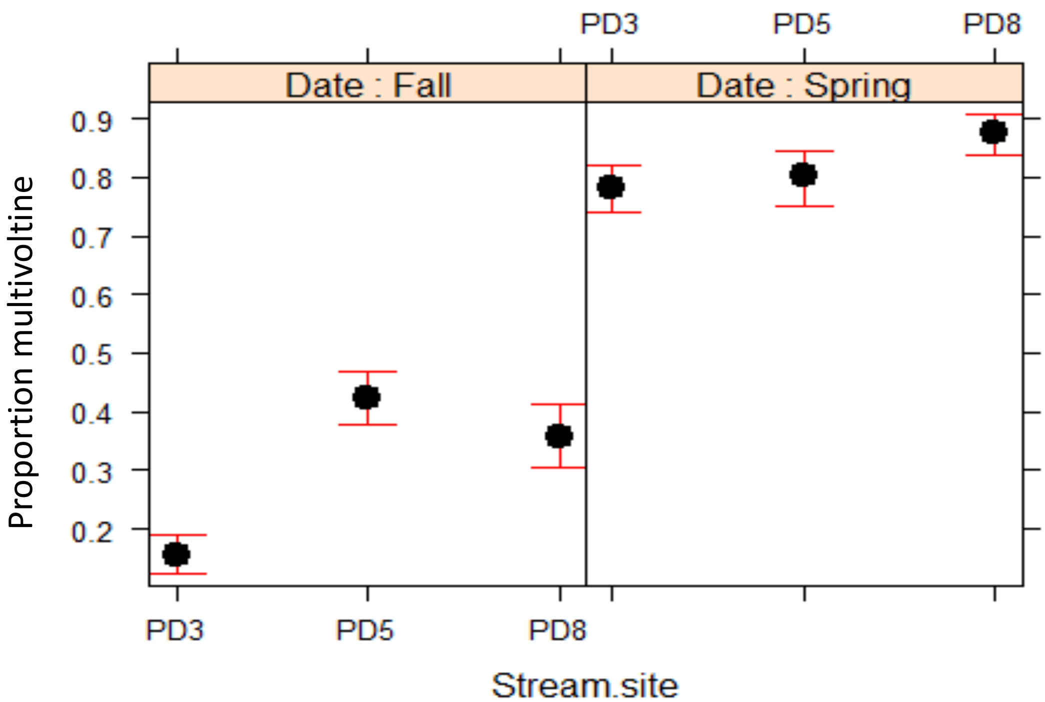

Figure 27. Mean proportions of the community comprised of individuals with a multivoltine life history, sampled in spring (April/May) and fall (September) 2016 from 3 sites in Pine Draw, TNWR.

Table 15. Logistic regression results for frequency of multivoltine life history among individuals collected from 3 sites on Pine Draw, spring and fall 2016.

	DF	Residual Dev	Dev	P
Null	29	863		
Site	2	45.6	818	<0.0001
Season	1	641	177	<0.0001
Site x Season	2	26	151	<0.0001

The proportion of the individuals with a wetland habitat affinity varied significantly by site, season, and with a site by season interaction (Table 15, Fig. 28). However, unlike HFBI, proportion dominant, and proportion multivoltine, the qualitative pattern of variation was similar between spring and fall, with PD3 having a much higher proportion of wetland taxa than PD5 or PD8. The proportion of wetland taxa in PD3 was higher in fall than spring. This pattern indicative of stressful stream conditions for aquatic assemblage during summer season compared to spring.

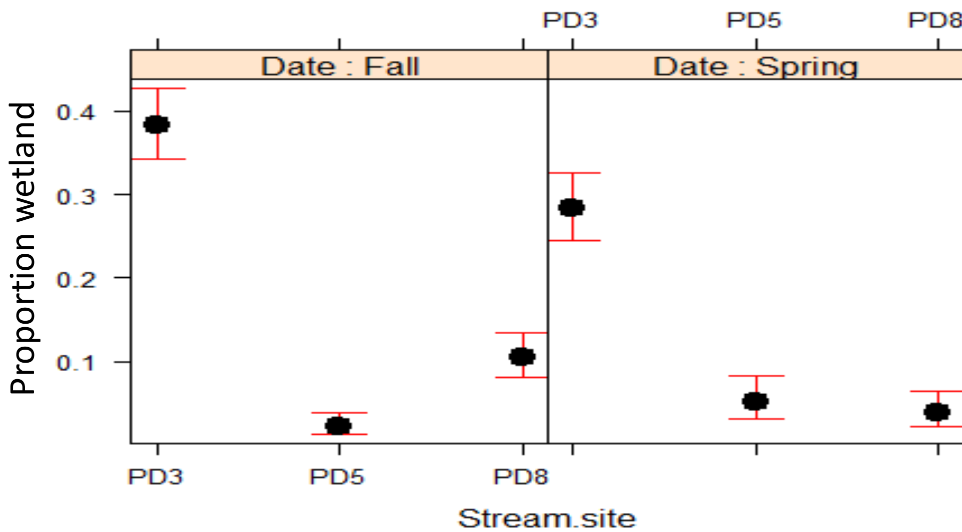

Figure 28. Mean proportions of the community comprised of individuals with wetland habitat affinity, sampled in spring (April/May) and fall (September) 2016 from 3 sites in Pine Draw, TNWR.

Table 16. Logistic regression results for frequency of wetland habitat affinity among individuals collected from 3 sites on Pine Draw, spring and fall 2016.

	DF	Residual Dev	Dev	P
Null	29	659		
Site	2	365	294	<0.0001
Season	1	14	279	<0.001
Site x Season	2	15	264	<0.001

Spring 2016 Analysis, 10 sites

HFBI varied significantly among sites, with CD3 having lower HFBI indicating higher water and habitat quality compared to the other sites in spring 2016 (Fig. 29, Table 16). Values for the 3 additional sites outside Company Ditch and Pine Draw (CT1, KD3, and PH3) had similar HFBI scores to most sites in Company Ditch and Pine Draw. CD3 site recorded higher water quality and greater integrity compared to CD4 which also had lower oxygen levels and higher dissolved nutrient concentrations based on the analyzed data.

HFBI did not differ between seasonal and permanent sites this year (Table 17). There is no obvious pattern in the results showing that seasonal and permanent sites consistently differ or have higher or lower water quality scores. This is an indication that the aspect of location was the most crucial factor when it comes to the question of whether a water source was of high quality or not. In other words, the question is not the length of time during which water is flowing in the stream but the nature of the surroundings that either enhance or reduce pollution of the water when it is flowing.

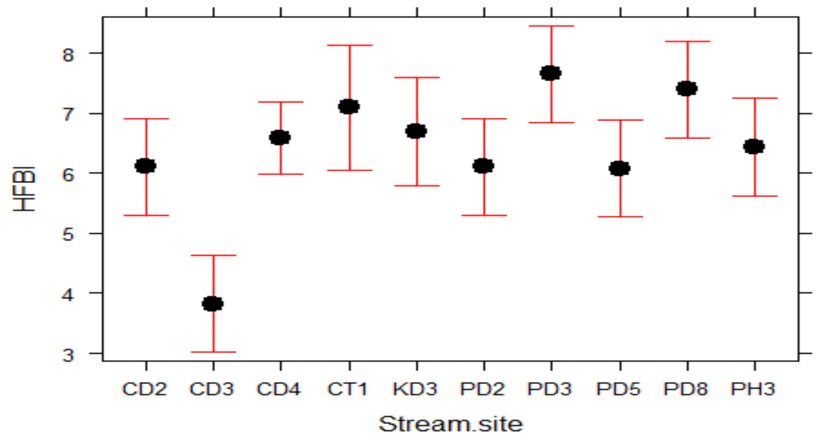

Figure 29. Mean HFBI scores for macroinvertebrate communities sampled from 10 sites on TNWR, spring 2016.

Table 17: One-way of HFBI scores for macroinvertebrate communities from 10 sites on TNWR, spring 2016.

	Df	SS	MS	F	P
Site	9	49.72	5.525	6.907	<0.0001
Residuals	41	32.80	0.800		

Table 18: One-way of HFBI scores for macroinvertebrate communities comparing seasonal and permanent stream sites on TNWR, spring 2016.

	Df	SS	MS	F	P
Hydroperiod	1	1.61	1.609	0.974	0.328
Residuals	49	80.91	1.651		

In contrast to HFBI, the frequency of the dominant taxon was highly variable among sites in spring 2016 (logistic regression, df = 9, $P < 0.0001$, Fig. 30). PH3 (Philleo Ditch) was found to have the highest proportion of the dominant taxon as compared to all other sites. On the other hand, CT1 was found to have relatively the same number of dominant taxa as CD3, PD2, PD3, and PD8. Dominant taxa values were lowest in one Company Ditch site (CD4).

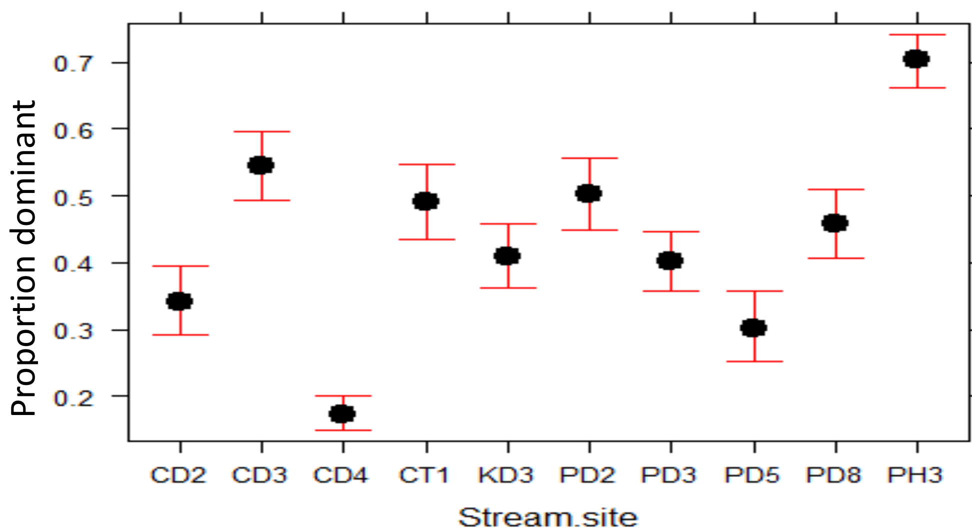

Figure 30. Mean proportion comprised of a single dominant taxon for macroinvertebrate communities sampled from 10 sites on TNWR, spring 2016.

Most sites were dominated by multivoltine individuals. (Fig. 31). However, there was significant variation in the frequency of multivoltine individuals within the macroinvertebrate communities of the different sites (logistic regression, d.f. = 9, $P < 0.0001$). CT1, downstream of Kepple Lake, had much lower frequency of multivoltine individuals than other sites. Frequency of multivoltine life history was also lower at the Keagle Ditch site (KD3).

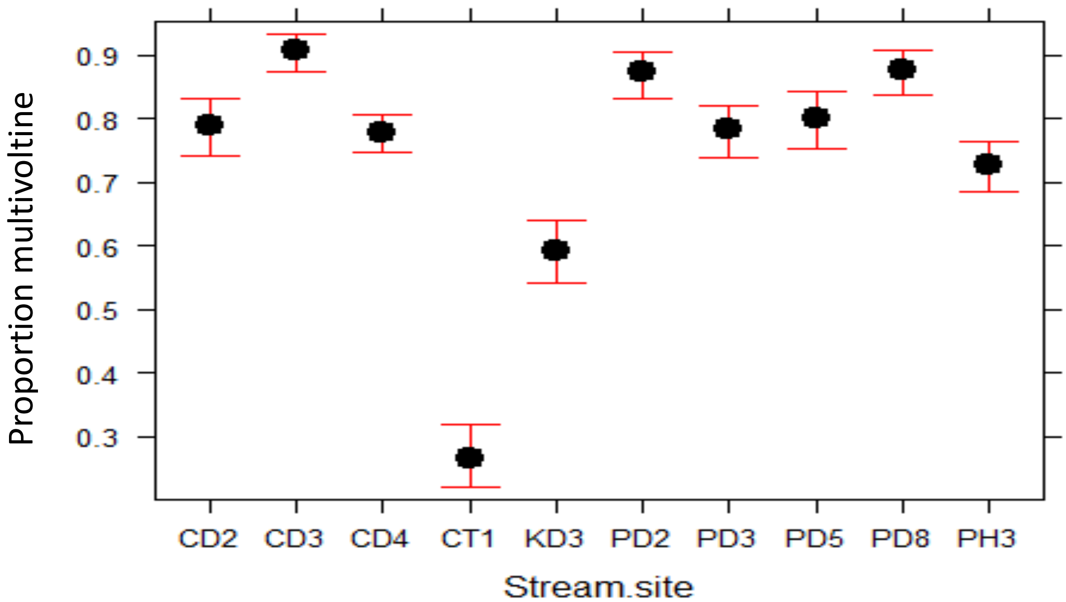

Figure 31. Mean proportion of individuals with a multivoltine life history strategy for macroinvertebrate communities sampled from 10 sites on TNWR, spring 2016.

Frequency of the individuals with a wetland habitat affinity was highly variable among sites in spring 2016 (logistic regression, df = 9, P < 0.0001, Fig. 32). CD2 and CT1 had the highest proportion of the wetland invertebrates compared to other sites. On the other hand, CD3, CD4, KD3, PD2, and PD3 had similar frequencies of wetland invertebrates. PD5 and PD8, the permanent stream sites, as well as PH3, had low frequencies of wetland taxa.

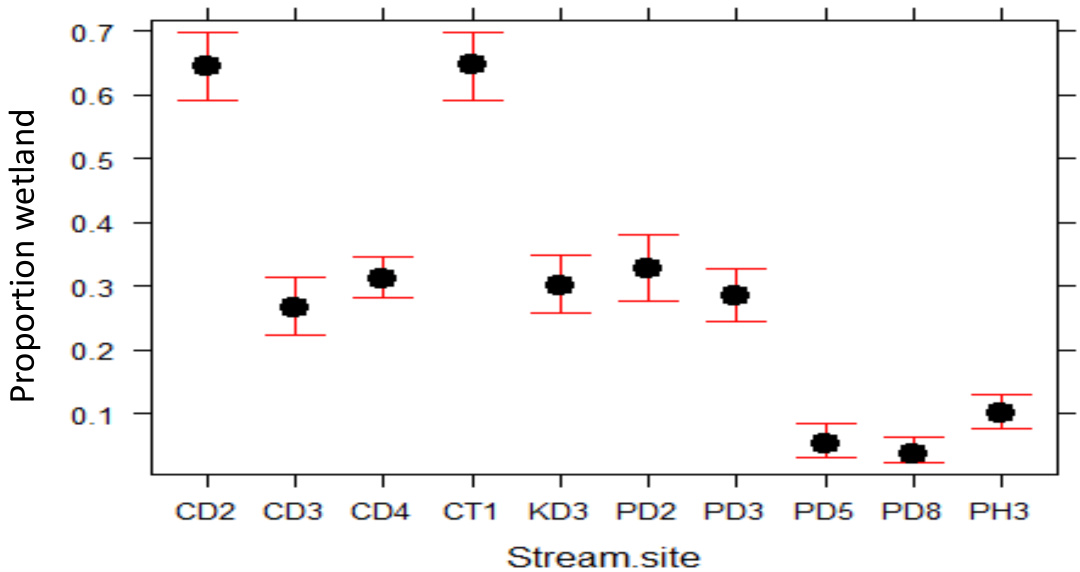

Figure 32. Mean proportion of individuals with a wetland habitat affinity for macroinvertebrate communities sampled from 10 sites on TNWR, spring 2016.

<u>Discussion</u>

The goal of this study was to evaluate a biological monitoring protocol for 6 stream sites on Turnbull National Wildlife Refuge, and to assess whether there were differences in water quality and ecosystem integrity among sites and over time. The study included two watersheds (Company Ditch and Pine Draw) and both seasonal and permanent stream sites.

Were there differences in water quality between seasonal and permanent stream sites?

For this question, I evaluated primarily 2016 data, as it included additional sites outside the two main watersheds. There were evident differences between the water quality of seasonal and permanent sites, in terms of pH and conductivity. Seasonal streams had lower pH and higher conductivity than the permanent sites. Higher conductivity in the seasonal sites likely reflects greater contribution of runoff to streamflow, more influence of evaporation, and may include septic influence at some sites. High pH in permanent Pine Draw sites may reflect high primary production in upstream wetlands reducing dissolved inorganic carbon concentrations. However, temperature, dissolved oxygen, and nutrient levels in the permanent sites were not consistently different from those of seasonal sites. These parameters are more likely to affect suitability of the habitat for invertebrates and other organisms. Most macroinvertebrate indices for permanent sites (HFBI, proportion dominant, proportion multivoltine) were not consistently different than those for seasonal sites, reflecting the lack of overall differences in water quality. However, in permanent sites, wetland taxa made up a smaller proportion of the community than for any seasonal sites, suggesting that the proportion of individuals with a wetland habitat affinity may be a reasonable hydrologic indicator.

Did water quality and ecosystem integrity vary among the sites?

All direct water quality measurements and macroinvertebrate indices that were statistically analyzed varied significantly among sites. There was also significant annual variation, and interactions between years and sites, making overall generalizations difficult. However, all Company Ditch sites consistently had highly levels of phosphate (SRP) compared to Pine Draw sites and the additional sites included in 2016. These high phosphate levels may cause eutrophication, contributing to low oxygen levels in some of the sites (all sites 2007, CD2 multiple years, CD4 2016). Low oxygen levels in CD3 have not been observed since 2007, however. HFBI index suggests substantially poorer water quality and/or ecosystem health in sites CD2 and CD4, than CD3. Examination of all macroinvertebrate metrics as well as the dominant taxa present in 2016 (Table 16) show that these three sites have distinct macroinvertebrate communities and have responded differently to changes through time in this watershed. The seasonal Pine Draw sites (PD2 and PD3) did not have elevated nutrient levels, and had more similar conductivity to permanent Pine Draw sites than to seasonal Company Ditch sites. In some ways invertebrate communities converged among CD3 and PD2 over the study period, and were similar to the Philleo (PH3) site sampled in 2016, which was also ephemeral. However, annual data indicate greater changes over time in seasonal sites compared to permanent sites.

How did water quality and ecosystem integrity change over the 10-year study period?

All water quality parameters and macroinvertebrate indices statistically analyzed varied with year and with year by site interaction. Temperature, pH, and stream depth (not statistically analyzed) appeared to vary substantially among years, with warmer water in 2013 and 2016. However, warmer water in these years was not consistently associated with low DO measured during the afternoon, when field work was conducted, perhaps due to high primary production at some of the sites.

Phosphate concentrations were consistently very high in Company Ditch sites from 2007 through 2010, and may be declining. A dairy located in the watershed closed in 2008; nutrient levels in the watershed may continue to decline as more time has elapsed since closure. From 2007-2008, Company Ditch sites were highly dominated by short-lived, pollution tolerant Ostracods. Beginning in 2010, reduced domination by Ostracods and increased diversity likely indicates improved water quality. Company Ditch site 3 (CD3) appears to have recovered more than the other two sites, as judged by macroinvertebrate indicators of water quality. CD2 is the most upstream site, and is likely highly influenced by human activity including residences with septic systems and livestock in the riparian zone just upstream and off the refuge. However, it is unclear why CD4, which is downstream of CD3 shows more limited recovery. Possible factors include conditions in the wetland just upstream of CD4, groundwater influences, and the deeply incised channel present at CD4.

Permanent Pine Draw sites PD5 and PD8 showed annual variation in some water quality and macroinvertebrate parameters, but no consistent changes through time. The seasonal Pine Draw site (PD2) appeared to maintain high water and habitat quality from 2008-2011, but had higher temperature, lower DO, and lower HFBI scores in 2013 and 2016. Future monitoring will help determine whether this represents annual variability or a long-term trend.

Would biological monitoring in spring vs. fall provide the same answer regarding relative ecosystem health of these streams?

Sampling for the long-term biological monitoring conducted by EWU's Freshwater Invertebrates course takes place during the spring, because this is when the primarily seasonal streams in the watersheds are flowing and contain macroinvertebrate communities. However, much macroinvertebrate bioassessment elsewhere in the northwestern U.S. takes place in September because that is when flows are lowest and streams are most accessible and safest for sampling. For permanent streams, results from September sampling may differ from spring sampling because stressful conditions during the summer months (warmer temperatures, lower DO, and in some cases more concentrated pollution with lower flows) will affect the invertebrate communities present. In addition, different invertebrate species have different timing of their life cycles within the year, which will alter community composition from spring to fall, even without changes in water quality.

In this study, water quality and macroinvertebrate parameters differed between spring and fall, and the pattern of variation among sites also differed between spring and fall. At two sites (PD3 and PD8) HFBI decreased between spring and fall, while at one site (PD5) HFBI increased from spring to fall. The dominance of the community by a single taxon decreased dramatically from spring to fall at PD8, but did not change at PD3. These responses indicate that bioassessment in spring may provide a different answer regarding the health of these stream communities, perhaps because summer conditions may impact some sites more than others. They also indicate that results of spring macroinvertebrate assessments should not be directly compared to fall assessments of streams outside TNWR.

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STREAMS	PLANT SPECIES
COMPANY DITCH	**Reed canary grass, hounds tongue, blue bunch grass, mullein, gallium snowberry, grasses, nettle, ponderosa pine, nightshade, bulrush, douglas fir, wood rose, balsamroot, *Ribes cercum*.**
PHILLIPS DITCH	**Reed canary grass, sedge, willow, other grass.**
PHILLEO DITCH	**Reed canary grass, nettle, other grass.**
KAEGLE DITCH	**Channel cleans incised, other grass, snowberry, pine, gallium.** **Water is coffee colored.**
CONTROL SITE	**Reed canary grass, thistle, aspen, cattail, water plant with yellow flowers.**

Appendix A. Plant species found at five streams Company ditch (CD), Phillips ditch (PD), Philleo ditch (PHE), Kaegle ditch (KD), and Control site (CT).

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EDUCATION

Bachelor of Science in Biology: January 2008 - August 2012
Jazan University, Jazan City, Saudi Arabia (KSA)
Bachelor degree in Biology GPA (4.12/5)

Intensive English Language Degree: August 2013 - September 2014
English Language Institute (ELI), Eastern Washington University (EWU), Cheney, WA
Completed intensive English language program at Eastern Washington University (EWU): skills reading, writing, listening, and speaking

EXPERIENCE

Working experience: January 2013 - March 2013
Biology Department of Jazan University, Jazan city, Saudi Arabia

- Faculty of Science in the biology department of Jazan University
- Assisted instructors during weekly invertebrate Zoology lab
- Worked in the office of biology department
- Prepared biological equipment's for Bio. Lab classes

Teaching Experience: May 2012 - September 2012
Teaching, Jeddah City, Saudi Arabia

- Taught at Cordobah High School, Jeddah City, Saudi Arabia.
- Gave advises for new students (Student Guide)
- Managed students for doing small science projects

Teaching Experience:

Department of Biology
Eastern Washington University
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- Teaching assistant at Eastern Washington University Bio 173-01

Sampling Experience: January 2015 - March 2016
Eastern Washington University, Cheney, WA

- Measured water quality (physical parameters)
- Used Rapid Bioassessment Protocol III for sampling and sorting aquatic species
- Sorted and Identified aquatic macroinvertebrate species to order, family, and GS
- Measured stream discharge

Statistical Analysis Experience: Jun 2014 - September 2014
Eastern Washington University, Cheney, WA

- Took three 500 statistical Analysis classes at EWU
- Used R Studio to make different kinds of figures
- Used Excel to make different kind of figures and ANOVA tables
- Organized and analyzed a long term dataset